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(54) Title: INTEGRIN ALPHA SUBUNIT CYTOPLASMIC DOMAIN POLYPEPTIDES, ANTIBODIES AND METHODS (57) Abstract Diagnostic systems, methods, polypeptides and antibodies for detecting the presence of the cytoplasmic domain of the in- tegrin α_{6B} or α_{3B} subunit in a body sample are disclosed.		

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1
INTEGRIN ALPHA SUBUNIT CYTOPLASMIC
DOMAIN POLYPEPTIDES, ANTIBODIES AND METHODS

Technical Field

5 The present invention relates to polypeptides that define the integrin α_6 and α_3 subunits, particularly the cytoplasmic domain of the α_6 and α_3 subunits. In addition, the invention describes antibodies immunoreactive with the cytoplasmic domain
10 of α_6 and α_3 , and methods for using the antibodies and polypeptides in assays for detecting α_6 and α_3 subunits in body samples.

Background

15 The integrin family of cell surface receptors serve cellular adhesion functions. The receptors form a link between the extracellular matrix and the cytoskeleton through their binding to various extracellular components. Each integrin receptor is a
20 heterodimer comprised of an α and a β subunit. At least 11 α chains (Ruoslahti and Giancotti, 1989) and six β chains (Sheppard et al., 1990) have been recognized in man. Each α subunit tends to associate with only one type of β subunit, but there are several
25 exceptions to this rule (Hemler et al., 1989; Cheresch et al., 1989; Holzmann et al., 1989; Freed et al., 1989).

 The human heterodimer VLA-6 was identified using the monoclonal antibody GoH3, which is immunoreactive
30 with the α_6 subunit expressed on the surface of mouse and human cells. Hemler et al. J. Biol. Chem., 263:7660-7665, (1988); and Sonnenberg et al. J. Biol. Chem., 262:10376-10383, (1987). The amino terminal sequence of the human VLA-6 α_6 subunit was determined
35 from purified protein (Kajiji et al. EMBO J, 8:673-680, 1989) and was used to design degenerate

oligonucleotides for probing a cDNA library. The full length sequence of α_6 cDNA, and its predicted amino acid sequence, were elucidated subsequent to cDNA cloning. Tamura, et al., J. Cell Biol., 111:1593-1604 (1990). While Tamura et al., supra, also disclose multiple cDNA sequences encoding the VLA-6 β_4 subunit, there is provided no evidence that additional VLA-6 α_6 subunits exist. European Patent Application Publication Number 279,669 (published July 24, 1988) describes human α_6 and β_4 subunits of an integrin receptor and the complex they associate to form on pancreatic and other cancer cells. The publication does not describe or suggest that an isoform of the α_6 subunit exists.

The full length sequence of a hamster cDNA encoding the Gap b3 cell surface membrane glycoprotein was described by Tsuji et al., J. Biol. Chem., 265:7016-7021 (1990). Based on the predicted amino acid sequence and predicted overall structure, it was suggested that Gap b3 is the hamster homolog of the α_3 integrin subunit. The sequence of a cDNA encoding the partial sequence of chicken α_3 protein was disclosed in Hynes et al. J. Cell Biol., 109:409-420 (1989). The cytoplasmic regions of these clones do not share homology with the cytoplasmic region of α_{3B} disclosed herein, and are therefore assumed to encode α_{3A} subunit isoform. Furthermore, neither publication suggest the possibility of an α_{3B} subunit.

The N-terminal amino acid sequence of human α_3 protein is provided in European Patent Application Publication Number 330,506 (published July 3, 1989). That publication provides no suggestion that an isoform of the α_3 protein, namely α_{3B} , exists.

35

Brief Summary of the Invention

A new species of alpha (α) integrin subunit protein has been discovered, with representative members in both the α_6 and α_3 class of integrins corresponding to the laminin receptor and the laminin, collagen and fibronectin receptors, respectively. Specifically, it has been discovered that new α_6 species and α_3 species exist which differ from previously described α_6 and α_3 proteins in the cytoplasmic domain of the protein. Through a combination of cDNA sequencing studies and anti-synthetic peptide antibody immunoreactivity studies, it has been shown that the cytoplasmic domain of these new proteins, designated α_{6B} and α_{3B} , are related between human and mouse isolates.

Thus the present invention describes polypeptides comprising an amino acid residue sequence that includes the amino acid residue sequence defining an antigenic determinant in the cytoplasmic domain of the human or mouse α_{6B} or α_{3B} protein. Preferably, the polypeptide has a sequence corresponding to the whole cytoplasmic domain of either the human or mouse α_{6B} or α_{3B} protein. Alternatively, a polypeptide can correspond to all or substantially all of a native human or mouse α_{6B} or α_{3B} subunit in substantially isolated form.

The polypeptides or proteins are useful as immunogens for preparing polyclonal and monoclonal antibodies immunoreactive with the human or mouse α_{6B} or α_{3B} cytoplasmic domains, and as reagents for use in diagnostic assays for detecting the α_{6B} or α_{3B} proteins.

Thus, in a related embodiment the invention describes polyclonal and monoclonal antibodies having immunospecificities for antigenic determinants on the cytoplasmic domains of α_{6B} and α_{3B} proteins. These antibodies find use in in vitro and in situ

immunoassays for detecting α_{6B} or α_{3B} cytoplasmic domain antigens in body samples such as tissues or fluids.

5 Another aspect of the invention is the diagnostic methods and kits therefor, for detecting α_{6B} or α_{3B} cytoplasmic domain antigenic determinants using an antibody of this invention.

10 Other features and benefits of the invention will become apparent from the following detailed description and specific examples describing the invention, its principles and preferred embodiments.

Brief Description of the Drawings

15 In the drawings forming a portion of this disclosure:

Figure 1 illustrates immunoprecipitation of polypeptides from mouse cells using antibodies specific for the α_6 subunit. The differentiated (Diff.) ES1 and D3 cells are described in Example 2. 20 Antibody GoH3 is a monoclonal antibody immunospecific for the extracellular domain of the α_{6A} subunit. Antisera 6844 was raised in rabbit against a synthetic peptide specific for the cytoplasmic domain of human α_{6A} . The immunoprecipitated labeled proteins were 25 visualized by SDS-PAGE. Molecular weight, in kilodaltons, is noted on the side of the gel.

Figures 2 and 3 illustrate a sequential immunoprecipitation analysis of α_6 subunits in human JAR cell lysates as described in Example 2. NRS is 30 normal rabbit preimmune sera, anti- α_6 Mab is GoH3, anti- α_{6A} is sera 6844 and anti- α_{6B} is sera 382. The molecular weight of standard protein markers is shown on the right side of the gel and is expressed in kilodaltons (KDa). Figure 2 shows immunodepletion 35 with NRS or with anti- α_6 Mab, and Figure 3 shows immunodepletion with anti- α_{6A} or with anti- α_{6B} .

Figure 4 shows α_{6A} and α_{6B} PCR amplification products visualized on an ethidium stained gel. Single-stranded cDNA was generated from human PG, JAR and U937 cells and was amplified with a set of primers, 1156 and 1157, specific for the human α_{6A} sequence as described in Example 3. The primers were also used to amplify the cloned human α_{6A} cDNA sequence, which yielded an amplification product of about 540 bp. The amplification products from the tested cell lines were either 540 bp or 410 bp, or both.

Figure 5 compares the nucleotide sequences of the 540 bp and 410 bp amplification products described in Figure 4. The 540 bp product shown on the top line is designated α_{6A} , and the 410 bp product shown on the bottom line is designated α_{6B} . Vertical bars denote where the two sequences are homologous. Horizontal dots denote a 130 nucleotide (nt) deletion in the α_{6B} sequence with respect to the α_{6A} sequence. The 130 nt deletion is in the region that encodes the α_{6A} cytoplasmic domain.

Figure 6 provides and compares the predicted amino acid sequence for the α_6 amplification products shown in Figure 5. The solid arrows show the location of the outer PCR primers; the broken arrows show the location of the nested inner PCR primers. The underlined sequence represent the putative transmembrane domain. The open boxed area is the α_{6A} cytoplasmic domain; the shaded boxed area is the α_{6B} cytoplasmic domain. The bracketed area represents the 130 nt sequence deleted from the α_{6B} sequence.

Figure 7 depicts an ethidium bromide-stained gel of the PCR amplification products generated from (A) undifferentiated ES1 and B16 cells and (B) undifferentiated and differentiated ES1 cells as described in Example 3. The same priming

oligonucleotides were used to amplify cDNA from these cells.

Figure 8 provides and compares the nucleotide and predicted amino acid sequences for the mouse α_6 amplification products shown in Figure 7. The α_{6B} sequence is on the top line; the α_{6A} sequence is on the bottom line. Predicted amino acid residues are noted below the nucleotide sequence. The solid arrows show the location of the PCR primers. The boxed regions encompass the start of cytoplasmic domain for the α_{6A} and α_{6B} proteins, respectively.

Figure 9 illustrates the results of in situ immunostaining of diseased human kidney tissue. Panel A is stained with polyclonal antisera 6488 specific for the α_{6A} cytoplasmic region. Panel B is stained with polyclonal antisera 382 specific for the α_{6B} cytoplasmic region.

Brief Description of the Sequences in the Sequence Listing

The Sequence Listing is shown after the Examples and before the Claims.

SEQ ID NO 1 is the 1073 residue amino acid sequence of the human α_{6A} protein. The putative transmembrane region is encompassed by amino acids 1012-1037. The mature protein is cleaved from the signal sequence between amino acids 23-24. Potential sites of N-linked glycosylation are at positions 223, 284, 370, 513, 731, 748, 891, 927 and 958. Putative cation binding domains are at positions 230-238, 324-332, 386-394 and 441-449. The cytoplasmic sequence GFFKR, which is conserved in virtually all of the integrin α chains, begins at amino acid position 1040. The sequence encoded by the fragment of α_{6A} cDNA amplified using primers 1156/1157 is encompassed by residues 927-1073.

SEQ ID NO 2 is the 5629 base nucleotide sequence of the human α_{6A} cDNA. The initiating ATG is at nucleotide position 147. The mature coding sequence begins at nucleotide position 216 and ends at position 3365. The cytoplasmic sequence GFFKR is encoded by nucleotides 3264-3278. The 130 nucleotide sequence present in SEQ ID NO 2 but deleted from SEQ ID NO 4 is encompassed by nucleotides 3261-3390. The sequence of the α_{6A} cDNA amplified using primers 1156/1157 is encompassed by nucleotides 2924-3455.

SEQ ID NO 3 is the 1091 residue amino acid sequence of the human α_{6B} protein. The sequence of SEQ ID NO 3 is identical to SEQ ID NO 1 between amino acids 1 and 1044. The sequence encoded by the fragment of α_{6B} cDNA amplified using primers 1156/1157 is encompassed by residue 927 through 1060.

SEQ ID NO 4 is the 5499 base nucleotide sequence of the human α_{6B} cDNA. The sequence of SEQ ID NO 4 is identical to SEQ ID NO 2 between nucleotides 1 and 3260. Nucleotides 3261-5499 of SEQ ID NO 4 are identical to nucleotides 3391-5629 of SEQ ID NO 2. SEQ ID NO 4 has a 130 nucleotide deletion in relation to SEQ ID NO 2. The sequence of the α_{6B} cDNA amplified using primers 1156/1157 is encompassed by nucleotides 2924-3325.

SEQ ID NO 5 is the 141 amino acid sequence predicted from the nucleic acid product which results from amplification of the mouse α_{6B} cDNA with primers 1157/1156. The putative transmembrane domain begins at amino acid 88 and continues through amino acid 113. SEQ ID NO 5 is identical to SEQ ID NO 7 at amino acid position 1 through 120; the two sequences diverge at amino acid 121.

SEQ ID NO 6 is the 426 base nucleotide sequence corresponding to the mouse α_{6B} amino acid sequence in SEQ ID NO 5. The putative transmembrane region is

encoded by nucleotides 262 through 337. SEQ ID NO 6 is identical to SEQ ID NO 8 except for 130 nucleotides present in SEQ ID NO 8 but deleted between nucleotides 342 and 343 of SEQ ID NO 6.

5 SEQ ID NO 7 is the 149 amino acid sequence predicted from the product which results from amplification of the mouse α_{6A} cDNA with primers 1157/1156. SEQ ID NO 7 is identical to SEQ ID NO 5 at amino acid positions 1 through 120; the sequences
10 diverge at amino acid 121.

 SEQ ID NO 8 is the 556 base nucleotide sequence corresponding to the mouse α_{6A} amino acid sequence in SEQ ID NO 7, plus the first 109 nucleotides in the 3' noncoding region. SEQ ID NO 8 is identical to SEQ ID
15 NO 6 except it has a 130 base insertion (nucleotides 342-472 of SEQ ID NO 8) between nucleotides 352 and 353 of SEQ ID NO 6.

 SEQ ID NO 9 is the 153 amino acid sequence predicted from the product which results from
20 amplification of the mouse α_{3B} cDNA with primers 2032/2033. The cytoplasmic sequence CDFFK begins at amino acid position 108.

 SEQ ID NO 10 is the 463 base nucleotide sequence corresponding to the mouse α_{3B} amino acid sequence in
25 SEQ ID NO 9. The cytoplasmic sequence CDFFK is encoded by nucleotides 324-338.

 SEQ ID NO 11 is the outer 5' PCR primer 1157, corresponding to bp 2918-2937 of the α_{6A} cDNA sequence of Sequence ID NO 2.

30 SEQ ID NO 12 is the outer 3' PCR primer 1156, corresponding to the complement of bp 3454-3473 of the α_{6A} cDNA sequence of SEQ ID NO 2.

 SEQ ID NO 13 is the inner 5' nested PCR primer 1681, corresponding to bp 2942-2960 of the α_{6A} cDNA
35 sequence of SEQ ID NO 2.

SEQ ID NO 14 is the inner 3' nested PCR primer 2002, corresponding to the complement of bp 3433-3452 of the α_{6A} cDNA sequence of SEQ ID NO 2.

5 SEQ ID NO 15 is the 5' PCR primer 2032, corresponding to the hamster α_{3A} cDNA sequence of Tsuji et al., J. Biol. Chem., 265:7016-7021 (1990).

SEQ ID NO 16 is the 3' PCR primer 2033, corresponding to the hamster α_{3A} cDNA sequence of Tsuji et al., J. Biol. Chem., 265:7016-7021 (1990).

10

Detailed Description of the Invention

A. Definitions

Amino Acid Residue: An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH₂ refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in J. Biol. Chem., 243:3552-59 (1969) and adopted at 37 C.F.R. 1.822(b)(2)), abbreviations for amino acid residues are shown in the following Table of Correspondence:

30

TABLE OF CORRESPONDENCE

<u>SYMBOL</u>		<u>AMINO ACID</u>
<u>1-Letter</u>	<u>3-Letter</u>	
Y	Tyr	tyrosine
G	Gly	glycine
35 F	Phe	phenylalanine
M	Met	methionine

	A	Ala	alanine
	S	Ser	serine
	I	Ile	isoleucine
	L	Leu	leucine
5	T	Thr	threonine
	V	Val	valine
	P	Pro	proline
	K	Lys	lysine
	H	His	histidine
10	Q	Gln	glutamine
	E	Glu	glutamic acid
	W	Trp	tryptophan
	R	Arg	arginine
	D	Asp	aspartic acid
15	N	Asn	asparagine
	C	Cys	cysteine

It should be noted that all amino acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and unusual amino acids, such as those listed in 37 C.F.R. 1.822(b)(4), and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates either a peptide bond to a further sequence of one or more amino acid residues or a covalent bond to a carboxyl or hydroxyl end group.

Polypeptide and Peptide: Polypeptide and peptide are terms used interchangeably herein to designate a linear series of amino acid residues connected one to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues.

Protein: Protein is a term used herein to designate a linear series of greater than about 50 amino acid residues connected one to the other as in a polypeptide.

5 Synthetic peptide: refers to a chemically produced chain of amino acid residues linked together by peptide bonds that is free of naturally occurring proteins and fragments thereof.

10 Nucleotide: A monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is a nucleoside. When the nucleoside
15 contains a phosphate group bonded to the 3' or 5' position of the pentose it is referred to as a nucleotide. A sequence of operatively linked nucleotides is typically referred to herein as a "base sequence" or "nucleotide sequence", and their
20 grammatical equivalents, and is represented herein by a formula whose left to right orientation is in the conventional direction of 5'-terminus to 3'-terminus.

Base Pair (bp): A partnership of adenine (A) with thymine (T), or of cytosine (C) with guanine (G)
25 in a double stranded DNA molecule. In RNA, uracil (U) is substituted for thymine.

Nucleic Acid: A polymer of nucleotides, either single or double stranded.

30 Polynucleotide: a polymer of single or double stranded nucleotides. As used herein "polynucleotide" and its grammatical equivalents will include the full range of nucleic acids. A polynucleotide will typically refer to a nucleic acid molecule comprised of a linear strand of two or more deoxyribonucleotides
35 and/or ribonucleotides. The exact size will depend on many factors, which in turn depends on the ultimate

conditions of use, as is well known in the art. The polynucleotides of the present invention include primers, probes, RNA/DNA segments, oligonucleotides or "oligos" (relatively short polynucleotides), genes, vectors, plasmids, and the like.

Gene: A nucleic acid whose nucleotide sequence codes for an RNA or polypeptide. A gene can be either RNA or DNA.

Duplex DNA: a double-stranded nucleic acid molecule comprising two strands of substantially complementary polynucleotides held together by one or more hydrogen bonds between each of the complementary bases present in a base pair of the duplex. Because the nucleotides that form a base pair can be either a ribonucleotide base or a deoxyribonucleotide base, the phrase "duplex DNA" refers to either a DNA-DNA duplex comprising two DNA strands (ds DNA), or an RNA-DNA duplex comprising one DNA and one RNA strand.

Recombinant DNA (rDNA) molecule: a DNA molecule produced by operatively linking two DNA segments. Thus, a recombinant DNA molecule is a hybrid DNA molecule comprising at least two nucleotide sequences not normally found together in nature. rDNA's not having a common biological origin, i.e., evolutionarily different, are said to be "heterologous".

Vector: a rDNA molecule capable of autonomous replication in a cell and to which a DNA segment, e.g., gene or polynucleotide, can be operatively linked so as to bring about replication of the attached segment. Vectors capable of directing the expression of genes encoding for one or more proteins are referred to herein as "expression vectors". Particularly important vectors allow cloning of cDNA (complementary DNA) from mRNAs produced using reverse transcriptase.

B. Integrin Alpha Subunit Polypeptides

The present invention relates to a previously undescribed species of integrin alpha subunit that is derived by splicing of the messenger RNA in the tissue in which the integrin alpha subunit is expressed, such that the amino acid sequence of the alpha subunit polypeptide has a sequence as defined herein.

Splicing as a form of regulation of gene expression is one means by which a cell regulates the structural gene products expressed in that cell type. According to the structures defined herein, it is now known that the α_6 and α_3 integrin subunits can each be expressed in two alternate forms (isoforms), designated herein as an "A" form and a "B" form depending upon the spliced product, and are referred to as α_{6A} or α_{6B} , and as α_{3B} or α_{3B} .

The newly described α_{6B} and α_{3B} subunits contain a carboxyterminal amino acid residue sequence defining their cytoplasmic domain that is different from their α_{6A} and α_{3A} counterparts. These new species of α_{6B} and α_{3B} provide, based on their sequence differences, novel polypeptide reagents based on (1) the antigenic determinants present in their cytoplasmic domains and (2) the structural role the cytoplasmic domain of these proteins play in the function of the integrins of which they are members.

1. α_{6B} Subunit Polypeptides

In one embodiment, the present invention contemplates a polypeptide based on the cytoplasmic domain of the α_{6B} species of the integrin α_6 subunit. This polypeptide has an amino acid sequence that includes a sequence that corresponds, and preferably is identical to, the amino acid residue sequence of the cytoplasmic domain of the human or mouse α_{6B} .

5 The cytoplasmic domain of human α_{6B} includes an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091 and of mouse α_{6B} has an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141.

10 Thus, in one embodiment, the present invention contemplates a polypeptide having an amino acid residue sequence that includes at least the sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091 that defines the carboxy terminal portion of cytoplasmic domain of human α_{6B} . Preferably a polypeptide has an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091, and more preferably has an amino acid residue sequence
15 shown in SEQ ID NO 3 from residue 1045 to residue 1091. In a related embodiment the invention contemplates the whole human α_{6B} protein, in a substantially isolated form, having a sequence shown in SEQ ID NO 3 from residue 1 to residue 1091.

20 By substantially isolated is meant that the protein is present in a composition as a major constituent, typically in amount greater than 10%, and preferably greater than 90%, of the total protein in the composition. Human α_{6B} protein can be isolated by
25 a variety of biochemical and immunological means from the tissue sources and cells described herein that contain α_{6B} subunit. Exemplary methods involve the use of a α_{6B} cytoplasmic domain specific antibody, such as 382 described herein, alone or in combination
30 with the teachings of Kajiji et al., EMBO J., 8:673-680 (1989).

In a related embodiment, the present invention contemplates a polypeptide having an amino acid residue sequence that includes at least the sequence
35 shown in SEQ ID NO 5 from residue 121 to residue 141 that defines a portion of the cytoplasmic domain of

mouse α_{6B} . Preferably a polypeptide has an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141. Also contemplated is the whole mouse α_{6B} protein in a substantially isolated form that included a sequence shown in SEQ ID NO 5 from residue 1 to residue 141, with the degree of isolation being the same as above for human α_{6B} . Purification of mouse α_{6B} can similarly be accomplished using the methods described above, and particularly using the murine cells described herein as a source of protein and an anti-peptide antibody prepared using mouse α_{6B} cytoplasmic domain-derived polypeptides.

The native mouse α_{6B} subunit polypeptide is a protein of about 125,000 daltons in molecular weight when analyzed by PAFF-SDS under reducing conditions as described in the Examples.

The native human α_{6B} subunit polypeptide is a protein of about 125,000 daltons in molecular weight when analyzed by polyacrylamide-sodium dodecyl sulfate gel electrophoresis (PAGE-SDS) under reducing conditions as described in the Examples.

2. α_{3B} Subunit Polypeptides

In another embodiment, the present invention contemplates a polypeptide based on the cytoplasmic domain of the α_{3B} species of the integrin α_{3B} subunit. This polypeptide has an amino acid sequence that includes a sequence that corresponds, and preferably is identical to, the amino acid residue sequence of the cytoplasmic domain of the human or mouse α_{3B} .

The cytoplasmic domain of mouse α_{3B} has an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153.

Thus, in one embodiment, the present invention contemplates a polypeptide having an amino acid residue sequence that includes at least the sequence shown in SEQ ID NO 9 from residue 113 to residue 153

that defines a portion of the cytoplasmic domain of α_{3B} . Preferably a polypeptide has an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153, and more preferably has an amino acid residue sequence shown in SEQ ID NO 9 from residue 1 to residue 153.

In a related embodiment, the invention contemplates the whole mouse α_{3B} protein, in a substantially isolated form having a sequence that includes the sequence shown in SEQ ID NO 9 from residue 1 to residue 153. The degree of isolation for mouse α_{3B} is the same as is for human α_{6B} above, with methods for preparing the mouse α_{3B} similarly based on immunoprecipitation or immunoaffinity isolation methods using an antibody specific for mouse α_{3B} cytoplasmic domain as defined herein.

In preferred embodiments, a polypeptide of the present invention comprises about 20 to 1100 amino acid residues, and preferably comprises about 24 to 50 amino acid residues.

Preferably, a polypeptide of this invention is further characterized by its ability to immunologically mimic an epitope (antigenic determinant) expressed by the cytoplasmic domain of α_{6B} or α_{3B} as defined herein.

As used herein, the phrase "immunologically mimic" in its various grammatical forms refers to the ability of a polypeptide of this invention to immunoreact with an antibody of the present invention that recognizes an epitope on the cytoplasmic domain of α_{6B} or α_{3B} as defined herein.

It should be understood that a subject polypeptide need not be identical to the amino acid residue sequence of α_{6B} or α_{3B} so long as it includes a sequence that provides at least one epitope within the cytoplasmic domain of the α_{6B} or α_{3B} subunit and is

able to immunoreact with antibodies of the present invention.

A subject polypeptide includes any analog, fragment or chemical derivative of a polypeptide whose amino acid residue sequence is shown herein so long as the polypeptide is capable of immunologically mimicking a native epitope present in the cytoplasmic domain of α_{6B} or α_{3B} . Therefore, a polypeptide can be subject to various changes, substitutions, insertions, and deletions where such changes provide for certain advantages in its use. The term "analog" includes any polypeptide having an amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the ability to mimic the cytoplasmic domain of α_{6B} or α_{3B} as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

The phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue provided that such polypeptide displays the requisite binding activity.

"Chemical derivative" refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include for example, those

molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. Polypeptides of the present invention also include any polypeptide having one or more additions and/or deletions or residues relative to the sequence of a polypeptide whose sequence is shown herein, so long as the requisite activity is maintained.

The term "fragment" refers to any subject polypeptide having an amino acid residue sequence shorter than that of a polypeptide whose amino acid residue sequence is shown herein.

When a polypeptide of the present invention has a sequence that is not identical to the sequence of the cytoplasmic domain of α_{5B} or α_{3B} because one or more conservative or non-conservative substitutions have been made, usually no more than about 30 number percent, more usually no more than 20 number percent, and preferably no more than 10 number percent of the amino acid residues are substituted, except that additional residues can be added at either terminus

for the purpose of providing a "linker" by which the polypeptides of this invention can be conveniently affixed to a label or solid matrix, or carrier, such that the linker residues do not form epitopes expressed by the cytoplasmic domain of α_{6B} or α_{3B} as defined herein. Labels, solid matrices and carriers that can be used with the polypeptides of this invention are described hereinbelow.

Amino acid residue linkers are usually at least one residue and can be 40 or more residues, more often 1 to 10 residues. Typical amino acid residues used for linking are tyrosine, cysteine, lysine, glutamic and aspartic acid, or the like. In addition, a subject polypeptide can differ, unless otherwise specified, from the natural sequence of an α_{6B} or α_{3B} cytoplasmic domain by the sequence being modified by terminal-NH₂ acylation, e.g., acetylation, or thioglycolic acid amidation, by terminal-carboxylamidation, e.g., with ammonia, methylamine, and the like.

When coupled to a carrier to form what is known in the art as a carrier-hapten conjugate, a polypeptide of the present invention is capable of inducing antibodies that immunoreact with the cytoplasmic domain of either human or mouse α_{6B} or mouse α_{3B} . Where the immunogen is an α_{3B} -derived polypeptide, the induced antibodies immunoreact with the cytoplasmic domain of either human or mouse α_{3B} . This cross-reactivity between human and mouse cytoplasmic domains is shown by the disclosures herein. In view of the well established principle of immunologic cross-reactivity, the present invention therefore contemplates antigenically related variants of the polypeptides of this invention. An "antigenically related variant" is a subject polypeptide that is capable of inducing antibody

molecules that immunoreact with a subject polypeptide and with α_{6B} or α_{3B} .

Any peptide of the present invention may be used in the form of a pharmaceutically acceptable salt.

5 Suitable acids which are capable of forming salts with the peptides of the present invention include inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acetic acid, propionic acid, 10 glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid or the like.

15 Suitable bases capable of forming salts with the peptides of the present invention include inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g. triethylamine, diisopropyl amine, methyl amine, 20 dimethyl amine and the like) and optionally substituted ethanolamines (e.g. ethanolamine, diethanolamine and the like).

A polypeptide of the present invention, also referred to herein as a subject polypeptide, can be 25 synthesized by any of the techniques that are known to those skilled in the polypeptide art, including recombinant DNA techniques. Synthetic chemistry techniques, such as a solid-phase Merrifield-type synthesis, are preferred for reasons of purity, 30 antigenic specificity, freedom from undesired side products, ease of production and the like. An excellent summary of the many techniques available can be found in J.M. Steward and J.D. Young, "Solid Phase Peptide Synthesis", W.H. Freeman Co., San Francisco, 35 1969; M. Bodanszky, et al., "Peptide Synthesis", John Wiley & Sons, Second Edition, 1976 and J. Meienhofer,

"Hormonal Proteins and Peptides", Vol. 2, p. 46, Academic Press (New York), 1983 for solid phase peptide synthesis, and E. Schroder and K. Kubke, "The Peptides", Vol. 1, Academic Press (New York), 1965 for classical solution synthesis, each of which is incorporated herein by reference. Appropriate protective groups usable in such synthesis are described in the above texts and in J.F.W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, New York, 1973, which is incorporated herein by reference.

In general, the solid-phase synthesis methods contemplated comprise the sequential addition of one or more amino acid residues or suitably protected amino acid residues to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively removable protecting group. A different, selectively removable protecting group is utilized for amino acids containing a reactive side group such as lysine.

Using a solid phase synthesis as exemplary, the protected or derivatized amino acid is attached to an inert solid support through its unprotected carboxyl or amino group. The protecting group of the amino or carboxyl group is then selectively removed and the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected is admixed and reacted under conditions suitable for forming the amide linkage with the residue already attached to the solid support. The protecting group of the amino or carboxyl group is then removed from this newly added amino acid residue, and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any

remaining terminal and side group protecting groups (and solid support) are removed sequentially or concurrently, to afford the final polypeptide.

5 An α_{68} or α_{38} -derived polypeptide can be used, inter alia, in the diagnostic methods and systems of the present invention to detect α_{68} or α_{38} present in a body sample, or can be used to prepare an inoculum as described herein for the preparation of antibodies that immunoreact with epitopes on the cytoplasmic
10 domain of either α_{68} or α_{38} .

C. DNA Segments

In living organisms, the amino acid residue sequence of a protein or polypeptide is directly related via the genetic code to the deoxyribonucleic
15 acid (DNA) sequence of the structural gene that codes for the protein. Thus, a structural gene can be defined in terms of the amino acid residue sequence, i.e., protein or polypeptide, for which it codes.

An important and well known feature of the
20 genetic code is its redundancy. That is, for most of the amino acids used to make proteins, more than one coding nucleotide triplet (codon) can code for or designate a particular amino acid residue. Therefore, a number of different nucleotide sequences may code
25 for a particular amino acid residue sequence. Such nucleotide sequences are considered functionally equivalent since they can result in the production of the same amino acid residue sequence in all organisms. Occasionally, a methylated variant of a purine or
30 pyrimidine may be incorporated into a given nucleotide sequence. However, such methylations do not affect the coding relationship in any way.

In one embodiment the present invention contemplates an isolated DNA segment that comprises a
35 nucleotide base sequence that encodes a polypeptide

that includes the amino acid residue sequence defining the cytoplasmic domain of α_{6B} or α_{3B} as defined herein.

A DNA segment therefor has a nucleotide sequence encoding the human or mouse α_{6B} or mouse α_{3B} proteins, or at least encoding the cytoplasmic domain of those proteins. The nucleotide sequences are generally shown in SEQ ID NO 4 for human α_{6B} , NO 6 for mouse α_{6B} and NO 10 for mouse α_{3B} .

Preferred DNA segments include a nucleotide base sequence represented by the base sequence contained in SEQ ID NO 4 from base 3279 to base 3418 and defining a coding sequence that translates into the cytoplasmic domain of α_{6B} . Particularly preferred is a nucleotide base sequence represented by the sequence contained in SEQ ID NO 4 from base 147 to base 3418 that defines the α_{6B} integrin subunit. Corresponding nucleotide sequences for mouse α_{6B} in SEQ ID NO 6 are also contemplated.

In another embodiment, preferred DNA segments include a nucleotide base sequence represented by the base sequence contained in SEQ ID NO 10 from base 339 to base 463 and defining a coding sequence that translates into the cytoplasmic domain of α_{3B} . Particularly preferred is a nucleotide base sequence represented by the sequence contained in SEQ ID NO 10 from base 1 to base 463 that defines the carboxy terminal portion of the α_{3B} integrin subunit, including the cytoplasmic domain of α_{3B} .

In preferred embodiments, the length of the nucleotide base sequence is no more than about 3,000 bases, preferably no more than about 1,000 bases.

A purified DNA segment of this invention is substantially free of other nucleic acids that do not contain the nucleotide base sequences specified herein for a DNA segment of this invention, whether the DNA segment is present in the form of a composition

containing the purified DNA segment, or as a solution suspension or particulate formulation. By substantially free is means that the DNA segment is present as at least 10% of the total nucleic acid present by weight, preferably greater than 50%, and more preferably greater than 90% of the total nucleic acid by weight.

In preferred embodiments, a DNA segment of the present invention is bound to a complementary DNA segment, thereby forming a double stranded DNA segment. In addition, it should be noted that a double stranded DNA segment of this invention preferably has a single stranded cohesive tail at one or both of its termini.

A DNA segment of the present invention can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci et al., J. Am. Chem. Soc., 103:3185 (1981). (The disclosures of the art cited herein are incorporated herein by reference.) Of course, by chemically synthesizing the structural gene portion, any desired modifications can be made simply by substituting the appropriate bases for those encoding a native amino acid residue.

In addition, a DNA segment can be prepared by first synthesizing oligonucleotides that correspond to portions of the DNA segment, which oligonucleotides are then assembled by hybridization and ligation into a complete DNA segment. Such methods are also well known in the art. See for example, Paterson et al., Cell, 48:441-452 (1987); and Lindley et al., Proc. Natl. Acad. Sci., 85:9199-9203 (1988), where a recombinant peptide, neutrophil-activated factor, was produced from the expression of a chemically synthesized gene in E. coli.

A DNA expression vector of the present invention is a recombinant DNA (rDNA) molecule adapted for

receiving and expressing translatable DNA sequences in the form of a fusion polypeptide of this invention. A DNA expression vector is characterized as being capable of expressing, in a compatible host, a structural gene product such as an α_{6B} or α_{3B} polypeptide of the present invention.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting between different genetic environments another nucleic acid to which it has been operatively linked. Preferred vectors are those capable of autonomous replication and expression of structural gene products present in the DNA segments to which they are operatively linked.

As used herein, the term "operatively linked", in reference to DNA segments, describes that the nucleotide sequence is joined to the vector so that the sequence is under the transcriptional and/or translation control of the expression vector and can be expressed in a suitable host cell.

The choice of vector to which a structural gene is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., vector replication and protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules.

In preferred embodiments, the vector utilized includes a prokaryotic replicon i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra chromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, those embodiments that include a prokaryotic replicon also include a gene whose expression confers a selective advantage, such as drug resistance, to a

bacterial host transformed therewith. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline. Vectors typically also contain convenient restriction sites for insertion of translatable DNA sequences. Exemplary vectors are the plasmids pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories, (Richmond, CA) and pPL and pKK223 available from Pharmacia, (Piscataway, NJ). Also contemplated are vectors for expressing a DNA segment of this invention in a yeast or mammalian host cell.

DNA expression control sequences include both 5' and 3' elements, as is well known, to form a cistron able to express a structural gene product. The 5' control sequences define a promoter for initiating transcription and a ribosome binding site operatively linked at the 5' terminus of the upstream translatable DNA sequence.

To achieve high levels of gene expression in *E. coli*, it is necessary to use not only strong promoters to generate large quantities of mRNA, but also ribosome binding sites to ensure that the mRNA is efficiently translated. In *E. coli*, the ribosome binding site includes an initiation codon (AUG) and a sequence 3-9 nucleotides long located 3-11 nucleotides upstream from the initiation codon [Shine et al., Nature, 254:34 (1975). The sequence, AGGAGGU, which is called the Shine-Dalgarno (SD) sequence, is complementary to the 3' end of *E. coli* 16S mRNA. Binding of the ribosome to mRNA and the sequence at the 3' end of the mRNA can be affected by several factors:

(i) The degree of complementarity between the SD sequence and 3' end of the 16S tRNA.

(ii) The spacing and possibly the DNA sequence lying between the SD sequence and the AUG [Roberts et

al., Proc. Natl. Acad. Sci. USA, 76:760 (1979a);
Roberts et al., Proc. Natl. Acad. Sci. USA, 76:5596
(1979b); Guarente et al., Science, 209:1428 (1980);
and Guarente et al., Cell, 20:543 (1980).]

5 Optimization is achieved by measuring the level of
expression of genes in plasmids in which this spacing
is systematically altered. Comparison of different
mRNAs shows that there are statistically preferred
10 sequences from positions -20 to +13 (where the A of
the AUG is position 0) [Gold et al., Annu. Rev.
Microbiol., 35:365 (1981)]. Leader sequences have
been shown to influence translation dramatically
(Roberts et al., 1979 a, b supra).

(iii) The nucleotide sequence following the AUG,
15 which affects ribosome binding [Taniguchi et al., J.
Mol. Biol., 118:533 (1978)].

D. Antibodies and Monoclonal Antibodies

The term "antibody" in its various grammatical
20 forms is used herein as a collective noun that refers
to a population of immunoglobulin molecules and/or
immunologically active portions of immunoglobulin
molecules, i.e., molecules that contain an antibody
combining site or paratope.

25 An "antibody combining site" is that structural
portion of an antibody molecule comprised of heavy and
light chain variable and hypervariable regions that
specifically binds (immunoreacts with) antigen. The
term immunoreact in its various forms means specific
30 binding between an antigenic determinant-containing
molecule and a molecule containing an antibody
combining site such as a whole antibody molecule or a
portion thereof.

The phrase "antibody molecule" in its various
35 grammatical forms as used herein contemplates both an

intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

Exemplary antibody molecules for use in the diagnostic methods and systems of the present invention are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the paratope, including those portions known in the art as Fab, Fab', F(ab')₂ and F(v).

Fab and F(ab')₂ portions of antibodies are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibodies by methods that are well known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous and Dixon. Fab' antibody portions are also well known and are produced from F(ab')₂ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules are preferred, and are utilized as illustrative herein.

An antibody of the present invention in one embodiment is an anti-cytoplasmic α_{63} domain antibody characterized as being capable of immunoreacting with 1) human α_{63} , and 2) a polypeptide having a sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.

In another embodiment an antibody of this invention is an anti-cytoplasmic α_{68} domain antibody characterized as being capable of immunoreacting with 1) human α_{68} , and 2) a polypeptide having a sequence shown in SEQ ID NO 3 from residue 1068-1091.

In another embodiment an antibody of this invention is an anti-cytoplasmic α_{68} domain antibody

characterized as being capable of immunoreacting with
1) mouse α_{6B} and 2) a polypeptide having a sequence
shown in SEQ ID NO 5 from residue 121 to residue 141.

5 In another embodiment, an anti-cytoplasmic α_{3B}
domain antibody is contemplated that is characterized
as being capable of immunoreacting with 1) mouse α_{3B} ,
and 2) the polypeptide having a sequence shown in SEQ
ID NO 9 from residue 113 to residue 153.

10 Antibody immunoreactivity with antigens
containing a cytoplasmic domain as defined above can
be measured by a variety of immunological assays known
in the art. Exemplary immunoreaction of a subject
antibody with α_{6B} or α_{3B} polypeptides is described in
Examples 2 and 4 .

15 For example, immunoreaction with whole protein
can be measured by the immunoprecipitation procedures
described in Example 2. Immunoreaction of antibodies
with polypeptides can be conveniently measured using
ELISA as described in U.S. Patents No. 3,643,090; No.
20 3,850,752; or No. 4,016,043, which are incorporated
herein by reference, using the polypeptide in the
solid phase, as is well know.

25 An antibody of the present invention is typically
produced by immunizing a mammal with an inoculum
containing a polypeptide of this invention and thereby
induce in the mammal antibody molecules having
immunospecificity for the polypeptide. Exemplary
immunization procedures for preparing an antibody of
this invention are described in Example 2. The
30 antibody molecules are then collected from the mammal
and isolated to the extent desired by well known
techniques such as, for example, by using DEAE
Sephadex to obtain the IgG fraction.

35 To enhance the specificity of the antibody, the
antibodies may be purified by immunoaffinity
chromatography using solid phase-affixed immunizing

polypeptide. The antibody is contacted with the solid phase-affixed immunizing polypeptide for a period of time sufficient for the polypeptide to immunoreact with the antibody molecules to form a solid phase-affixed immunocomplex. The bound antibodies are separated from the complex by standard techniques.

The antibody so produced can be used, inter alia, in the diagnostic methods and systems of the present invention to detect α_{6B} or α_{3B} subunits present in a body sample. See, for example, the methods described in Examples 2 and 4.

The word "inoculum" in its various grammatical forms is used herein to describe a composition containing a polypeptide of this invention as an active ingredient used for the preparation of antibodies against the cytoplasmic domain of an α_{6B} or α_{3B} polypeptide. When a polypeptide is used in an inoculum to induce antibodies it is to be understood that the polypeptide can be used in various embodiments, e.g., alone or linked to a carrier as a conjugate, or as a polypeptide polymer. However, for ease of expression and in context of a polypeptide inoculum, the various embodiments of the polypeptides of this invention are collectively referred to herein by the term "polypeptide", and its various grammatical forms.

For a polypeptide that contains fewer than about 35 amino acid residues, it is preferable to use the peptide bound to a carrier for the purpose of inducing the production of antibodies.

One or more additional amino acid residues can be added to the amino- or carboxy-termini of the polypeptide to assist in binding the polypeptide to a carrier. Cysteine residues added at the amino- or carboxy-termini of the polypeptide have been found to be particularly useful for forming conjugates via

disulfide bonds. However, other methods well known in the art for preparing conjugates can also be used.

Exemplary additional linking procedures include the use of Michael addition reaction products, di-
5 aldehydes such as glutaraldehyde, Klipstein, et al., J. Infect. Dis., 147:318-326 (1983) and the like, or the use of carbodiimide technology as in the use of a water-soluble carbodiimide to form amide links to the carrier. For a review of protein conjugation or
10 coupling through activated functional groups, see Aurameas, et al., Scand. J. Immunol., 1:7-23 (1978).

Useful carriers are well known in the art, and are generally proteins themselves. Exemplary of such carriers are keyhole limpet hemocyanin (KLH), edestin,
15 thyroglobulin, albumins such as bovine serum albumin (BSA) or human serum albumin (HSA), red blood cells such as sheep erythrocytes (SRBC), tetanus toxoid, cholera toxoid as well as polyamino acids such as poly (D-lysine: D-glutamic acid), and the like.

20 The choice of carrier is more dependent upon the ultimate use of the inoculum and is based upon criteria not particularly involved in the present invention. For example, a carrier that does not generate an untoward reaction in the particular animal
25 to be inoculated should be selected.

The present inoculum contains an effective, immunogenic amount of a polypeptide of this invention, typically as a conjugate linked to a carrier. The effective amount of polypeptide per unit dose
30 sufficient to induce an immune response to the immunizing polypeptide depends, among other things, on the species of animal inoculated, the body weight of the animal and the chosen inoculation regimen as is well known in the art. Inocula typically contain
35 polypeptide concentrations of about 10 micrograms to about 500 milligrams per inoculation (dose),

preferably about 50 micrograms to about 50 milligrams per dose.

The term "unit dose" as it pertains to the inocula refers to physically discrete units suitable as unitary dosages for animals, each unit containing a predetermined quantity of active material calculated to produce the desired immunogenic effect in association with the required diluent; i.e., carrier, or vehicle. The specifications for the novel unit dose of an inoculum of this invention are dictated by and are directly dependent on (a) the unique characteristics of the active material and the particular immunologic effect to be achieved, and (b) the limitations inherent in the art of compounding such active material for immunologic use in animals, as disclosed in detail herein, these being features of the present invention.

Inocula are typically prepared from the dried solid polypeptide-conjugate by dispersing the polypeptide-conjugate in a physiologically tolerable (acceptable) diluent such as water, saline or phosphate-buffered saline to form an aqueous composition.

Inocula can also include an adjuvant as part of the diluent. Adjuvants such as complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA) and alum are materials well known in the art, and are available commercially from several sources.

The techniques of polypeptide conjugation or coupling through activated functional groups presently known in the art are particularly applicable. See, for example, Aurameas, et al., Scand. J. Immunol., Vol. 8, Suppl. 7:7-23 (1978) and U.S. Patent No. 4,493,795, No. 3,791,932 and No. 3,839,153. In addition, a site directed coupling reaction can be carried out so that any loss of activity due to

polypeptide orientation after coupling can be minimized. See, for example, Rodwell et al., Biotech., 3:889-894 (1985), and U.S. Patent No. 4,671,958.

5 One or more additional amino acid residues may be added to the amino- or carboxy-termini of the polypeptide to assist in binding the polypeptide to form a conjugate. Cysteine residues, usually added at the carboxy-terminus of the polypeptide, have been
10 found to be particularly useful for forming conjugates via disulfide bonds, but other methods well-known in the art for preparing conjugates may be used.

A preferred antibody of this invention is a monoclonal antibody.

15 The phrase "monoclonal antibody" in its various grammatical forms refers to a population of antibody molecules that contain only one species of antibody combining site capable of immunoreacting with a particular epitope. A monoclonal antibody thus
20 typically displays a single binding affinity for any epitope with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different epitope, e.g., a
25 bispecific monoclonal antibody.

A monoclonal antibody is typically composed of antibodies produced by clones of a single cell called a hybridoma that secretes (produces) but one kind of antibody molecule. The hybridoma cell is formed by
30 fusing an antibody-producing cell and a myeloma or other self-perpetuating cell line. The preparation of such antibodies was first described by Kohler and Milstein, Nature 256:495-497 (1975), which description is incorporated by reference. The hybridoma
35 supernates so prepared can be screened for the presence of antibody molecules that immunoreact with a

polypeptide of this invention, or for inhibition of the natural function of an α_{6B} or α_{3B} subunit.

Briefly, to form the hybridoma from which the monoclonal antibody composition is produced, a myeloma
5 or other self-perpetuating cell line is fused with lymphocytes obtained from the spleen of a mammal hyperimmunized with an antigen containing the cytoplasmic domain of α_{6B} or α_{3B} , such as is present in a polypeptide of this invention. The polypeptide-
10 induced hybridoma technology is described by Niman et al., Proc. Natl. Sci., U.S.A., 80:4949-4953 (1983), which description is incorporated herein by reference.

It is preferred that the myeloma cell line used to prepare a hybridoma be from the same species as the
15 lymphocytes. Typically, a mouse of the strain 129 Glx⁺ is the preferred mammal. Suitable mouse myelomas for use in the present invention include the hypoxanthine-aminopterin-thymidine-sensitive (HAT) cell lines P3X63-Ag8.653, and Sp2/0-Ag14 that are
20 available from the American Type Culture Collection, Rockville, MD, under the designations CRL 1580 and CRL 1581, respectively.

Splenocytes are typically fused with myeloma cells using polyethylene glycol (PEG) 1500. Fused
25 hybrids are selected by their sensitivity to HAT. Hybridomas producing a monoclonal antibody of this invention are identified using an immunoassay such as the immunoprecipitation protocol described in Example 3.

30 A monoclonal antibody of the present invention can also be produced by initiating a monoclonal hybridoma culture comprising a nutrient medium containing a hybridoma that secretes antibody molecules of the appropriate polypeptide specificity.
35 The culture is maintained under conditions and for a time period sufficient for the hybridoma to secrete

the antibody molecules into the medium. The antibody-containing medium is then collected. The antibody molecules can then be further isolated by well known techniques.

5 Media useful for the preparation of these compositions are both well known in the art and commercially available and include synthetic culture media, inbred mice and the like. An exemplary synthetic medium is Dulbecco's minimal essential
10 medium (DMEM; Dulbecco et al., Virology 8:396 (1959)) supplemented with 4.5 gm/1 glucose, 20 mm glutamine, and 20% fetal calf serum. An exemplary inbred mouse strain is the Balb/c.

15 The monoclonal antibodies of this invention can be used in the same manner as disclosed herein for antibodies of the present invention.

For example, the monoclonal antibody can be used in the diagnostic methods and systems disclosed herein where formation of a cytoplasmic α_{6B} or α_{3B} domain-
20 containing immunoreaction product is desired.

Other methods of producing a monoclonal antibody, a hybridoma cell, or a hybridoma cell culture are also well known. See, for example, the method of isolating monoclonal antibodies from an
25 immunological repertoire as described by Sastry, et al., Proc. Natl. Acad. Sci., 86:5728-5732 (1989); Huse et al., Science, 246:1275-1281 (1989); and Mullinax et al, Proc.Natl.Acad.Sci.USA, 87:8095-8099 (1990).

Also contemplated by this invention is the
30 hybridoma cell, and cultures containing a hybridoma cell that produce a monoclonal antibody of this invention.

D. Diagnostic Systems

35 The present invention also describes a diagnostic system, preferably in kit form, for assaying for the presence of antigen having the cytoplasmic domain of

α_{6B} or α_{3B} in a body sample such as a tissue, body fluid or the like body sample. A diagnostic system includes, in an amount sufficient for at least one assay, a subject polypeptide and/or a subject antibody or monoclonal antibody, as a separately packaged immunochemical reagent. Instructions for use of the packaged reagent are also typically included.

As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil and the like capable of holding within fixed limits a polypeptide, polyclonal antibody or monoclonal antibody of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated polypeptide or it can be a microtiter plate well to which microgram quantities of a contemplated polypeptide have been operatively affixed, i.e., linked so as to be capable of being immunologically bound by an antibody.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions and the like.

In one embodiment, a diagnostic system for assaying for the presence of or to quantitate α_{6B} or α_{3B} in a sample, such as blood, plasma or serum, comprises a package containing at least one α_{6B} or α_{3B} derived polypeptide of this invention depending on whether α_{6B} or α_{3B} is to be detected, respectively. In another embodiment, a diagnostic system of the present invention for assaying for the presence or amount of α_{6B} or α_{3B} in a sample further includes an antibody

composition of this invention. An exemplary diagnostic system is described in Example 4.

5 In preferred embodiments, a diagnostic system of the present invention further includes a label or indicating means capable of signaling the formation of an immunocomplex containing a polypeptide or antibody molecule of the present invention.

10 The word "complex" as used herein refers to the product of a specific binding reaction such as an antibody-antigen or receptor-ligand reaction. Exemplary complexes are immunoreaction products.

15 As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of a complex. Any label or indicating means can be linked to or incorporated in an expressed protein, polypeptide, or antibody molecule that is part of an antibody or monoclonal antibody composition of the present invention, or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel proteins methods and/or systems.

20 The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC),

25 30 35

lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis, et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

In preferred embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, or the like. In such cases where the principal indicating group is an enzyme such as HRP or glucose oxidase, additional reagents are required to visualize the fact that a receptor-ligand complex (immunoreactant) has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with glucose oxidase is 2,2'-azino-di-(3-ethyl-benzthiazoline-G-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as ^{124}I , ^{125}I , ^{128}I , ^{132}I and ^{51}Cr represent one class of gamma ray emission-producing radioactive element indicating groups. Particularly preferred is ^{125}I . Another group of useful labeling means are those elements such as ^{11}C , ^{18}F , ^{15}O and ^{13}N which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, such as ^{111}In indium of ^3H .

The linking of labels, i.e., labeling of, polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of

radioisotope-containing amino acids provided as a component in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). The techniques of protein conjugation or coupling through activated functional groups are particularly applicable. See, for example, Aurameas, et al., Scand. J. Immunol., Vol. 8 Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S. Pat. No. 4,493,795.

The diagnostic systems can also include, preferably as a separate package, a specific binding agent. A "specific binding agent" is a molecular entity capable of selectively binding a reagent species of the present invention or a complex containing such a species, but is not itself a polypeptide or antibody molecule composition of the present invention. Exemplary specific binding agents are second antibody molecules, complement proteins or fragments thereof, S. aureus protein A, and the like. Preferably the specific binding agent binds the reagent species when that species is present as part of a complex.

In preferred embodiments, the specific binding agent is labeled. However, when the diagnostic system includes a specific binding agent that is not labeled, the agent is typically used as an amplifying means or reagent. In these embodiments, the labeled specific binding agent is capable of specifically binding the amplifying means when the amplifying means is bound to a reagent species-containing complex.

The diagnostic kits of the present invention can be used in an "ELISA" format to detect the quantity of α_{6B} or α_{3B} subunit in a vascular fluid sample such as blood, serum, or plasma. "ELISA" refers to an enzyme-linked immunosorbent assay that employs an antibody or antigen bound to a solid phase and an enzyme-antigen

or enzyme-antibody conjugate to detect and quantify the amount of an antigen present in a sample. A description of the ELISA technique is found in Chapter 22 of the 4th Edition of Basic and Clinical Immunology by D.P. Sites et al., published by Lange Medical Publications of Los Altos, CA in 1982 and in U.S. Patents No. 3,654,090; No. 3,850,752; and No. 4,016,043, which are all incorporated herein by reference.

Thus, in preferred embodiments, a polypeptide or an antibody of the present invention can be affixed to a solid matrix to form a solid support that comprises a package in the subject diagnostic systems.

A reagent is typically affixed to a solid matrix by adsorption from an aqueous medium although other modes of affixation applicable to proteins and polypeptides well known to those skilled in the art, can be used.

Useful solid matrices are also well known in the art. Such materials are water insoluble and include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose; beads of polystyrene beads about 1 micron to about 5 millimeters in diameter available from Abbott Laboratories of North Chicago, IL; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a microtiter plate such as those made from polystyrene or polyvinylchloride.

The reagent species, labeled specific binding agent or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be

provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

The packaging materials discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems.

The term "package" refers to a solid matrix or material such as glass, plastic (e.g., polyethylene, polypropylene and polycarbonate), paper, foil and the like capable of holding within fixed limits a diagnostic reagent such as a polypeptide, antibody or monoclonal antibody of the present invention. Thus, for example, a package can be a bottle, vial, plastic and plastic-foil laminated envelope or the like container used to contain a contemplated diagnostic reagent or it can be a microtiter plate well to which microgram quantities of a contemplated diagnostic reagent have been operatively affixed, i.e., linked so as to be capable of being immunologically bound by an antibody or polypeptide to be detected.

F. Assay Methods

The present invention contemplates various immunoassay methods for determining the amount of α_{6B} or α_{3B} in a biological sample using a polypeptide, polyclonal antibody or monoclonal antibody of this invention as an immunochemical reagent to form an immunoreaction product whose amount relates, either directly or indirectly, to the amount of α_{6B} or α_{3B} in the sample.

Those skilled in the art will understand that there are numerous well known clinical diagnostic chemistry procedures in which an immunochemical reagent of this invention can be used to form an immunoreaction product whose amount relates to the

amount of α_{68} or α_{38} present in a body sample. Thus, while exemplary assay methods are described herein, the invention is not so limited.

5 Various heterogeneous and homogeneous protocols, either competitive or noncompetitive, can be employed in performing an assay method of this invention, including radioimmunoprecipitation (RIP), solid phase immunoassay such as ELISA, in situ immunoreaction assays for direct binding of antigen in tissue
10 samples, and the like immunoassay protocols.

Generally, to detect the presence of an α_{68} or α_{38} subunit or polypeptide in a patient, an aliquot (i.e., a predetermined amount) of a body fluid sample, such as urine or a vascular fluid, namely blood,
15 plasma or serum from the patient, or a tissue sample prepared for immunoreaction, is contacted by admixture (admixed), with an antibody composition of the present invention to form an immunoreaction admixture. The admixture is then maintained under biological assay
20 conditions (immunoreaction conditions) for a period of time sufficient for the α_{68} or α_{38} antigen present in the sample to immunoreact with (immunologically bind) a portion of the antibody combining sites present in the antibody composition to form a antigen-antibody
25 molecule immunoreaction product (immunocomplex). The complex can then be detected as described herein. The presence of the complex is indicative of α_{68} or α_{38} subunit or polypeptide in the sample.

Maintenance time periods sufficient for
30 immunoreaction are well known and are typically from about 10 minutes to about 16-20 hours at a temperature of about 4°C to about 45°C, with the time and temperature typically being inversely related. For example, longer maintenance times are utilized at
35 lower temperatures, such as 16 hours at 4°C, and

shorter times for higher temperatures, such as 1 hour at room temperature.

Biological assay conditions are those that maintain the biological activity of the immunochemical reagents of this invention and the α_{6B} or α_{3B} subunit or polypeptide sought to be assayed such that the reagents retain their ability to form an immunoreaction product. Those conditions include a temperature range of about 4°C to about 45°C, a pH value of about 5 to about 9 and an ionic strength varying from that of distilled water to that of about one molar sodium chloride. Methods for optimizing such maintenance time periods and biological assay conditions are well known in the art.

Determining the presence or amount of an α_{6B} or α_{3B} containing immunoreaction product formed by the above maintenance step, either directly or indirectly, can be accomplished by assay techniques well known in the art, and typically depend on the type of indicating means used.

In a direct binding assay format for detecting α_{6B} or α_{3B} in a tissue sample such as a tissue section, the antibody is reacted with the target antigen in situ to form the immunoreaction complex. thereafter, the immunocomplex is detected thereby indicating the presence of the antigen in the tissue. Exemplary and preferred in situ immunoassay formats are described in Example 4. Alternatively, the direct binding assay can be practiced with a fluid body sample believed to contain α_{6B} or α_{3B} subunits or polypeptides.

Thus, in this embodiment, the direct assay comprises the steps of:

(a) admixing a tissue sample or body fluid sample with an antibody composition of this invention immunospecific for a cytoplasmic domain of either α_{6B}

or α_{3B} as described herein to form an immunoreaction admixture;

(b) maintaining said immunoreaction admixture under biological assay conditions for a time period sufficient to form an immunoreaction product; and

(c) detecting the presence, and preferably amount, of the immunoreaction product formed phase in step (b), and thereby the amount of presence/amount of α_{6B} or α_{3B} in the sample.

More preferably, detecting in step (c) is performed by the steps of:

(i) admixing the immunoreaction product formed in step (b) with an indicating means to form a second reaction admixture;

(ii) maintaining the second reaction admixture for a time period sufficient for said indication means to bind the immunoreaction product formed in step (b) and form a second reaction product; and,

(iii) determining the presence and/or amount of indicating means in the second reaction product, and thereby the presence of the immunoreaction product formed in step (b). Particularly preferred is the use of a labeled second antibody, immunospecific for the first antibody, as the indicating means, and preferably the label is horseradish peroxidase. In one embodiment, it is particularly preferred to use (1) mouse anti-cytoplasmic domain α_{6B} polypeptide antibody in the antibody composition, and (2) goat anti-mouse IgG antibodies labeled with horseradish peroxidase as the indicating means.

In a preferred competition assay method, the immunoreaction admixture described above further contains a solid phase having affixed thereto a solid phase antigen comprising an α_{6B} or α_{3B} subunit or polypeptide having an amino acid residue sequence that includes the cytoplasmic domain of α_{6B} or α_{3B} ,

respectively, of this invention. Thus, in this embodiment, the assay comprises the steps of:

5 (a) admixing a body fluid sample with 1) an antibody composition of this invention and 2) a solid support having affixed thereto (operatively linked) an antigen comprising an α_{6B} or α_{3B} subunit or polypeptide having an amino acid residue sequence that includes the cytoplasmic domain of α_{6B} or α_{3B} of this invention, or both, to form an immunoreaction admixture having
10 both a liquid phase and a solid phase;

(b) maintaining said immunoreaction admixture under biological assay conditions for a time period sufficient to form an immunoreaction product in the solid phase; and

15 (c) detecting the presence, and preferably amount, of the immunoreaction product formed in the solid phase in step (b), and thereby the amount of presence/amount of one or both of α_{6B} and α_{3B} in the body fluid sample.

20 In another competition assay format the immunoreaction admixture contains (1) a body fluid sample, preferably cell free, (2) an antibody of this invention and (3) a labeled antigen comprising the cytoplasmic domain of α_{6B} or α_{3B} , wherein the antibody
25 is present in the solid phase, being affixed to a solid support, to form a liquid and a solid phase. In this embodiment, the admixed body fluid sample competes with the labeled reagent for immunoreaction with the solid phase antibody to form a solid phase
30 immunoreaction product. Thereafter, the detection of label in the solid phase correlates with the amount of α_{6B} or α_{3B} in the admixed fluid sample.

In one embodiment, the detection of a polypeptide of this invention in a body sample is utilized as a
35 means to monitor the fate of therapeutically

administered α_{6B} or α_{3B} derived polypeptides according to the therapeutic methods disclosed herein.

Also contemplated are immunological assays capable of detecting the presence of immunoreaction product formation without the use of a label. Such methods employ a "detection means", which means are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel polypeptides, methods and systems. Exemplary detection means include methods known as biosensors and include biosensing methods based on detecting changes in the reflectivity of a surface, changes in the absorption of an evanescent wave by optical fibers or changes in the propagation of surface acoustical waves.

Examples

The following Examples illustrate, but do not limit, the present invention.

1. Polypeptides

Polypeptides were synthesized using the classical solid-phase technique described by Merrifield, Adv. Enzymol., 32:221-96 (1969) as adapted for use with a Model 430A automated peptide synthesizer (Applied Biosystems, Foster City, CA). Polypeptide resins were cleaved by hydrogen fluoride, extracted and analyzed for purity by high-performance liquid chromatograph using a reverse-phase C18 column. (Waters Associates, Milford, MA).

The amino acid residue sequence of the polypeptides and their designations are as follows:

$p\alpha_{6A}1$ IHAQPSDKERLTSDA

$p\alpha_{6B}1$ DEKYIDNLEKKQWITKWNRNESYS

Polypeptide $p\alpha_{6A}$ has a sequence from the cytoplasmic domain of α_{6A} and is shown in SEQ ID NO 1

from residue 1059 to residue 1073 to which an additional cysteine residue was included at the N-terminus for coupling the peptide to a protein carrier (KLH) for immunization. Polypeptide $\text{p}\alpha_{6B}$ has a sequence from the cytoplasmic domain of α_{6B} and is shown in SEQ ID NO 3 from residue 1068 to residue 1091 to which an additional cysteine residue was included at the N-terminus for coupling the peptide to a protein carrier (KLH) for immunization.

2. Preparation of Polyclonal Antisera

a. Conjugation of KLH

Briefly, as a generalized procedure for each polypeptide, 4 milligrams of KLH in 0.25 milliliters (ml) of 10 millimolar (mM) sodium phosphate buffer (pH 7.2) is reacted with 0.7 milligrams (mg) of MBS dissolved in DMF, and the resulting admixture is stirred for 30 minutes at room temperature. The MBS solution is added dropwise to ensure that the local concentration of DMF was not too high, as KLH is insoluble at DMF concentrations of about 30% or higher. The reaction product, KLH-MB, is passed through a chromatography column prepared with Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with 50 mM sodium phosphate buffer (pH 6.0) to remove free MBS. KLH recovery from peak fractions of the column eluate, monitored at 280 nanometers, is typically approximately 80%.

The KLH-MB so prepared is then reacted with 5 mg of polypeptide dissolved in 1 ml of buffer. The pH value of the resulting reaction composition is adjusted to 7-7.5, and the reaction composition is stirred at room temperature for 3 hours to provide a polypeptide-carrier conjugate.

b. Immunization and Harvesting of Polyclonal Antisera

Inoculum stock solutions are prepared with CFA, IFA or alum as follows: An amount of the synthetic polypeptide-conjugate sufficient to provide the desired amount of polypeptide per inoculation is dissolved in phosphate-buffered saline (PBS) at a pH value of 7.2. Equal volumes of CFA, IFA or alum are then mixed with the polypeptide solution to provide an inoculum containing polypeptide, water and adjuvant in which the water-to-oil ratio is about 1:1. The mixture is thereafter homogenized to provide the inoculum stock solution.

Rabbits used herein to raise anti-polypeptide antibodies were injected subcutaneously with an inoculum comprising 200 micrograms (ug) of a polypeptide conjugate (polypeptide plus carrier) emulsified in complete Freund's adjuvant (CFA); 200 ug of polypeptide conjugate, incomplete in Freund's adjuvant (IFA); and 200 ug of polypeptide conjugate with 4 mg alum injected intraperitoneally on days 0, 14 and 21, respectively, of the immunization schedule. Each inoculation (immunization) consisted of four injections of the inoculum. Mice may be immunized in a similar way using about one tenth of the above dose per injection.

Animals are typically bled 4 and 15 weeks after the first injection. Control re-immune serum was obtained from each animal by bleeding just before the initial immunization.

Control inoculum stock solutions can also be prepared with keyhole limpet hemocyanin (KLH), KLH in IFA (incomplete Freund's adjuvant), KLH-alum absorbed, KLH-alum absorbed-pertussis, edestin, thyroglobulin, tetanus toxoid, tetanus toxoid in IFA, cholera toxoid and cholera toxoid in IFA.

Upon injection or other introduction of the antigen or inoculum into the host animal, the immune

system of the animal responds by producing large amounts of antibody to the antigen. Since the specific antigenic determinant of the manufactured antigen; i.e., the antigen formed from the synthetic polypeptide linked to the carrier corresponds to the determinant of the natural antigen of interest, the host animal manufactures antibodies not only to the synthetic polypeptide to which the synthetic polypeptide antigen corresponds; i.e., to the α_{6B} protein.

c. Immunoreactivity of Anti-peptide Antisera With Native α_6 Proteins

1. Protocols and Reagents

The rabbit polyclonal anti- α_6 cytoplasmic domain antiserum designated 6844 was raised against the last 15 amino acids (IHAQPSDKERLTSDA) (SEQ ID NO 1, residue 1059 to residue 1073) of the reported human α_6 (α_{6A}) sequence (Tamura et al., J. Cell. Biol., 111:1593-1604, 1990), to which an additional cystine residue was included at the N-terminus for coupling the peptide to a protein carrier (KLH) for immunization.

The rat monoclonal antibody, GoH3, is specific for an extracellular epitope on both the human and murine α_6 subunits (Sonnenberg et al., J. Biol. Chem., 262:10376-83, 1987). The isotype-matched control antibody, B3B4, recognizes the B lymphocyte specific antigen, CD23.

The anti- α_6 specific monoclonal antibody, 135.13c, and the isotype matched control antibody, 439.9b, specific for the human β_4 integrin subunit, have been previously described (Kennel et al., J. Biol. Chem., 264:15515-21, 1989).

Anti-peptide antisera to the cytoplasmic domains of rat α_1 , chicken α_3 , human α_1 , human α_3 , and human β_1 sequences were shown to be cross-reactive with the

respective mouse β_1 integrins by immunoprecipitation of B16F1 melanoma, STO fibroblast and MMT carcinoma murine cell lines.

Antisera to the cytoplasmic domain of human α_{6B} were prepared by immunizations of rabbits with the peptide $p\alpha_{6B}1$ having the sequence DEKYIDNLEKKQWITKWNRNESYS (SEQ ID NO 3, residue 1068 to residue 1091) as described above to which an additional cysteine residue was included at the N-terminus for coupling the peptide to a protein carrier (KLH) for immunization. This antisera is designated 382.

The ES cells and B16F1 cell line were used in these immunoreaction studies. The ES cell line, CCE (Schwartzberg et al., Science, 246:799-803, 1989) was initially cultured on murine embryonic fibroblasts (STO cells) to prevent differentiation. However, in order to study the expression and function of integrins in this ES cell line it was necessary to remove the STO cells from the culture system. Therefore, the CCE ES cell line was subcloned into LIF (10^3 units/ml) (Amrad Co. Australia) containing media (DMEM; 10% FCS, 100mM β -mercaptoethanol, 2mM glutamine). LIF has been shown to prevent ES cell differentiation (Moreau et al., Nature, 336:690-92, 1988; Smith et al., Nature, 336:688-90, 1988; Williams et al., Nature, 336:684-687, 1988). The sublines were cultured on gelatin (0.1%) coated plates. Several subclones were expanded and continually cultured in LIF containing media. The subline ES1 was chosen for the studies described here. ES1 cells were allowed to differentiate on gelatin (0.1%) coated plates over a period of 8-9 days in the absence of LIF.

The murine B16F1 melanoma line, obtained from Dr. Ralph Reisfeld (Scripps Clinic, La Jolla, California), was derived from a C57Bl/6 melanoma and cultured in

DMEM, 5% FCS, 2mM glutamine and penicillin-streptomycin (50 IU/ml- 50ug/ml).

5 Undifferentiated ES cells ($1-2 \times 10^7$ cells) were surface labeled with Na^{125}I using the lactoperoxidase procedure (Roth, Methods Enzymol., 37(B):223-33, 1975). Differentiated ES cells proved to be significantly more fragile than undifferentiated ES cells and did not survive the more rigorous washing steps required during the iodination procedure.

10 Therefore, differentiated ES cells were metabolically labeled with [^{35}S]methionine as described previously by Kajiji et al, EMBO J., 8:673-680 (1989).

Preparation of non-ionic detergent cell lysates, immunoprecipitations and analysis by SDS-PAGE were performed as described by Kajiji et al (1989), supra.

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Immunoprecipitation is conducted generally by first admixing the rabbit polyclonal antisera produced above with a cell lysate and maintaining the admixture for a time period sufficient for immunocomplexes to form. Thereafter, the immunoabsorbent Pansorbin (Sigma Chemical Co., St. Louis, MO) is added to the admixture containing the immunocomplexes and maintained to allow the Pansorbin to complex with (bind) the immunocomplex. Thereafter the Pansorbin-containing bound immunocomplexes are removed from the lysate admixture by centrifugation, washed several times and the washed immunocomplexes are released from the Pansorbin and analyzed by SDS-PAGE.

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Sequential immunoprecipitation was also performed to identify the presence of multiple immunoreactive species in a single lysate. After a first immunoprecipitation as above the lysate is retained and subjected to a second immunoprecipitation with unbound Pansorbin. The resulting lysate from the second immunoprecipitation is again retained and subjected to a third immunoprecipitation with unbound

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Pansorbin. Thus by the successive rounds of sequential immunoprecipitation of a lysate using the same antibody species, that lysate becomes depleted of antigen immunoreactive with that antibody species. Thereafter, the depleted lysate is divided into aliquots and each aliquot is separately immunoprecipitated (re-IP or re-immunoprecipitated) using different antibodies. Antigens in the depleted lysate that immunoprecipitate with the second antibody different from the depleting first antibody are not immunoreactive with the first antibody. By sequential immunoprecipitation, two non-cross reacting antigen species can be identified. As described herein, the cytoplasmic domains of α_{6A} and α_{6B} are not cross reactive.

Mouse α_6

Separate immunoprecipitations were carried out on undifferentiated murine ES1 cells with antiserum 382 raised against a synthetic peptide corresponding to the sequence of the cytoplasmic tail of human α_{6B} , with control preimmune serum from the same rabbit, and with antisera 6844 directed to the cytoplasmic tail of human 6A. Only antisera 382 precipitated protein bands virtually identical to those reactive with anti- α_6 monoclonal GoH3 which is specific for both α_{6A} and α_{6B} . These data indicated that ES1 cells do express α_{6B} protein, probably complexed with β_1 , and antisera 382 is capable of recognizing the α_{6B} protein.

In contrast to the immunoprecipitation data from undifferentiated ES1 cells, the anti- α_{6A} cytoplasmic domain polyclonal antiserum, 6844, could immunoprecipitate the α_{6A} isoform from ^{35}S -methionine labelled lysates obtained from differentiated ES1 cells. Thus, differentiation of ES1 cells is

accompanied by the induction of expression of the α_{6A} isoform.

5 The absence of the α_{6A} isoform in differentiated ES1 cells can be seen by immunoprecipitations using GoH3 or 6844, which is shown in Figure 1. Whereas the GoH3 antibody detects the lower molecular weight species corresponding to α_6 , the 6844 antibody, immunospecific for α_{6A} , does not detect any α_6 species, indicating that the GoH3-reactive form is an isoform, namely α_{6B} .

10 Similar immunoprecipitation assays were carried out on the D3 ES cell line (see Figure 1). The D3 embryonic stem cell line was derived by Doetschman et al., J. Embryol. Exp. Morph., 87:27-45, (1985). D3 cells were cultured in LIF containing medium as described above except that 15% FCS was used. Immunoprecipitations of [35 S]methionine-labelled lysates showed that the α_{6B} isoform is expressed at the protein level in both undifferentiated and differentiated D3 cells while the α_{6A} isoform was found only in the differentiated cells. This would suggest that the ability to switch on α_{6A} expression upon differentiation may be a general property of ES cells.

25 Because the 382 antisera was raised to a human α_{6B} cytoplasmic domain-derived polypeptide and yet is shown above to immunoreact with the mouse α_{6B} protein, the above data also shows that an anti- α_{6B} antibody, whether raised to human or mouse varieties of α_{6B} can be used to immunoreact with both human or mouse α_{6B} .

Human α_6

Antisera 382 to a synthetic peptide corresponding to the last 25 residues of human α_{6B} immunoprecipitated from radiolabeled detergent lysates of the human choriocarcinoma cell line JAR (see Example 4 for description of JAR cells) a pattern of

bands similar or identical to those obtained with 6844, an anti-peptide antiserum to the α_{6A} cytoplasmic domain, and GoH3, a monoclonal antibody to the extracellular domain of α_6 (see Figure 2). The bands corresponded in molecular weight to α_6 , β_1 and β_4 , and were positively identified as such with specific antibodies. This result is compatible with JAR cells expressing both $\alpha_6\beta_1$ and $\alpha_6\beta_4$ heterodimers, and with PCR amplifications detecting both α_{6A} and α_{6B} isoform bands in JAR cells (see Example 3).

Sequential immunoprecipitations (Figure 3) showed that antibody GoH3 completely depleted the JAR lysates of antigen reactive with antisera 382 (anti- α_{6B}) or 6844 (anti- α_{6A}). The 382 antiserum did not remove any material reactive with 6844, and 6844 did not remove any 382-reactive material, while both antisera reduced, but did not completely remove GoH3 reactivity (Figure 2). These results indicate that JAR cells express both α_{6A} and α_{6B} proteins, each of which is paired with either β_1 or β_4 . These results also indicate that antisera raised to a mouse protein, namely the cytoplasmic domain of mouse α_{6B} , immunoreacts with its human counterpart protein, human α_{6B} .

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3. Identification and Cloning of α_{6B} and α_{3B} cDNAs

cDNA molecules encoding human and mouse α_{6B} and mouse α_{3B} cytoplasmic regions were prepared and fragments of the cDNA molecules were selectively amplified using the polymerase chain reaction (PCR) in the presence of specific oligonucleotide primers in order to characterize gene expression of the α_{6B} and α_{3B} proteins.

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a. Procotols

Poly-A+ mRNA was isolated from human JAR cells (American Tissue Type Collection, ATCC, Bethesda, MD, ATCC HTB 144), human U937 cells (ATCC CRL 1591), human FG cells (Dr. P. Meitner, Brown University) and both differentiated and undifferentiated cell lines using the Invitrogen Fastrack Kit (Invitrogen, La Jolla, Ca.). Single stranded cDNA was synthesized from 10 ug of mRNA using AMV reverse transcriptase (20U; Molecular Genetics Resources, Tampa, Fl.) and one ug of random hexamer primers (Pharmacia). The cDNAs were extracted with phenol/chloroform, then ethanol precipitated and about 0.5 to 10 ug cDNA was resuspended in 50-70ul of sterile water.

One ul of the resuspended cDNA was amplified per 50 ul PCR reaction mixture (2.5mM MgCl₂, 50mM KCl, 10mM β -mercaptoethanol, 66mM Tris.HCl; pH8.3) using 0.1 uM oligonucleotide primers, 0.25mM each of dATP, dTTP, dCTP, and dGTP, and 1.25U of TAQ 1 polymerase (AmpliTaQ; Perkin Elmer/Cetus, Ca.). The PCR program consisted of 2 steps: (a) 40 cycles of 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C with a 5 sec/cycle extension on the 72°C segment, (b) 10 min at 72°C and a final shift to 4°C. Second round PCR was carried out on one ul of the reaction mixture generated from the first round PCR.

Nested pairs of PCR primers were employed to ensure that α_6 specific fragments were amplified. Both sets of α_6 primers were derived from the human α_{6A} cDNA sequence as determined by Tamura et al., J. Cell. Biol., 111:1593-1604, (1990). The first set corresponded to bp 2918-2937 (primer 1157) and 3454-3473 (primer 1156) of the human α_{6A} sequence while the nested primer pair corresponded to bp 2942-2960 (primer 1681) and 3433-3452 (primer 2002). The sequence of these four primers are shown in SEQ ID NOS

11-14, respectively. α_3 PCR primers designated primer 2032 and 2033 were derived from the hamster cDNA sequence as determined by Tsuji et al., J. Biol. Chem., 265:7016-7021 (1990). The sequence of primers 2032 and 2033 are shown in SEQ ID NOS 15 and 16, respectively.

Oligonucleotide primers were chemically synthesized by using a "Gene Assembler" automated synthesizer (Pharmacia, Piscataway, NJ).

Amplified fragments from first round PCR were purified using Gene Clean (Bio 101, La Jolla, Ca), treated with DNA polymerase I and T4 polynucleotide kinase, again purified with Gene Clean, and the blunt-ended fragments were subcloned into the EcoRV site of Bluescript-pKS+ (Stratagene, La Jolla, Ca). Clones containing insert were sequenced manually (Sequenase kit; USB, Cleveland, Oh) according to the manufacturer's instructions using T3 and T7 polymerase vector primer sequences. Sequences were analyzed on a VAX-VMS, version 5.2 computer, with programs of the University of Wisconsin Genetics Computer Group (Devereux et al, Nucl. Acids Res., 12:387-395, 1984).

b. Results

i. Human α_6

Oligonucleotides 1156 and 1157 flanking the 3' end of the coding region of integrin α_6 mRNA were used as primers in polymerase chain reactions (PCR). Two products, of 540 bp and 410 bp, were obtained using first strand cDNAs from various cell lines as templates (Figure 4). These same products were obtained in second-round PCR with a nested set of primers, indicating their specificity.

Both the 540bp and the 410bp PCR products were subcloned and sequenced. The nucleotide sequence of the 540 bp fragment (designated α_{6A} in

Figure 5) matches exactly the sequence of α_6 mRNA recently reported by Tamura et al., J. Cell. Biol., 111:1593-1604, 1990, and encodes the 3' portion of the end of the extracellular domain, the transmembrane and the cytoplasmic domains, followed by the initial part of the 3' untranslated region (3' UT).

The sequence of the 410 bp band matches the 540 bp sequence, with the exception of a 130 bp gap shown in the lower sequence of Figure 5, which lower sequence corresponds to the nucleotide sequence of α_{6B} . This gap corresponds to the region encoding the predicted α_{6A} cytoplasmic domain, from the boundary with the transmembrane domain to 25 bp past the stop codon. Without this 130 bp segment, however, the open reading frame continues in the previous 3' UT, resulting in an α_{6B} protein with an alternative cytoplasmic domain (Figure 6). This alternative domain is 17 amino acid longer than, and bears no sequence homology with, the reported α_{6A} cytoplasmic domain, but it does contain the sequence GFFKR, a motif present at the upstream border of all mammalian integrin α chains sequenced. For convenience, the published α_6 sequence is referred to as α_{6A} , and the α_6 having the isoform cytoplasmic domain identified herein is referred to as α_{6B} .

ii. Mouse α_6

Amplification of mouse α_6 cDNA

expressed by undifferentiated ES1 and B16F1 cells was performed on first strand cDNA derived from these mouse cells using the polymerase chain reaction (PCR). The nested sets of PCR primers, pairs 1157/1156 and 1681/2002 described above, were employed. Figure 7A shows the PCR products obtained.

The PCR fragment amplified from B16F1 ("B16") cDNA correspond to the size expected (510 bp) for the murine homologue of the human α_6 (Figure 7A; lane 2).

However, the PCR fragment obtained from the amplification of the ES1 cell cDNA was significantly smaller. Amplification of cDNAs derived from four independent ES1 mRNA preparations yielded only the smaller fragment and never the larger fragment amplified from B16F1 cDNA.

The PCR fragments from the ES1 and B16F1 cells were subcloned into the Bluescript-pKS+ vector and sequenced. Figure 8 shows the nucleotide sequences of the two PCR fragments. The sequence of the larger B16F1 fragment was shown to be 89% identical to the human α_{6A} sequence at the nucleotide level and 91% identical at the amino acid level, Tamura et al., J. Cell. Biol., 111:1593-1604 (1990). Thus the larger fragment's sequence represents the murine homologue of the human α_{6A} subunit. The B16F1 PCR fragment (Figure 8) encodes the C-terminal portion of the extracellular domain as well as the transmembrane and cytoplasmic domains of the α_6 subunit. Due to the selection of primers, additional coding sequences 3' to the terminus of the sequence shown in Figure 8 were not detected. Thus, additional amino acid residues not shown in Figure 8 are present in the native mouse α_{6B} protein.

The sequence of the smaller PCR fragment (Figure 8) was identical to the B16F1 sequence except that an internal deletion of 130 bp was observed. The location of the 130 bp deletion observed in the ES1 α_6 PCR fragment exactly matched that of the human α_{6B} sequence. Therefore, ES1 cells expressed the murine equivalent of the α_{6B} isoform.

iii. Mouse α_3

Expression of the α_3 isoforms was also investigated in various mouse tissues including muscle, heart, brain, lung and ovary. Using the PCR procedure described above with the hamster α_3 primers,

a larger band corresponding to α_{3A} was amplified from most tissues except heart, kidney, liver, thymus and spleen (Table 2; Example 4). A smaller band corresponding to α_{3B} was detected in heart and brain. Cloning and sequencing of these bands showed that the larger band corresponds exactly to the reported α_3 sequence (α_{3A}), while the smaller band lacks a 144 bp segment and, like α_{6B} , encodes an α_3 with an alternative cytoplasmic tail (α_{3B}). The amino acid residue and nucleotide sequences of the mouse α_{3B} cDNA-derived PCR fragments are shown in SEQ ID Nqs 9 and 10, respectively.

4. Tissue distribution of α_{6A} , α_{6B} , α_{3A} and α_{3B}

a. PCR Amplification

The distribution of the α_6 isoforms in cultured cell lines and mouse tissue was assessed by PCR as described in Example 3. The majority of the cells tested contained both α_{6A} and α_{6B} mRNA (see Tables 1 and 2). However, the two isoforms were reproducibly found at ratios characteristic of a cell line. Interestingly, two carcinoma cell lines and three lines of mouse embryonic fibroblasts (immortalized, non-transformed) contained exclusively α_{6A} , while embryonic stem cells and F9 teratocarcinoma cells contained exclusively α_{6B} (Table 1).

TABLE 1

<u>CELL LINE</u>	<u>CELL TYPE</u>	<u>α_{6A}</u>	<u>α_{6B}</u>	<u>α_{3A}</u>	<u>α_{3B}</u>
FG	Pancreatic Carcinoma	+	>	+	+
1320 Met	"	+	>	+	ND
Panc-1	"	+	>	+	+
SGR	"	+	>	+	ND
JAR	Choriocarcinoma	+	<	+	+
JEG-3	"	+	<	+	ND

	BeWo	"	+	<	+	ND	ND
	LoVo	Colon Carcinoma	+	<	+	ND	ND
	Colo 396	"	+	<	+	+	-
	CaCo-2	"	+		+	+	-
5	HT-29	"	+	>	+	+	-
	HeLa	Cervical Carcinoma	+	>	+	ND	ND
	UCLA-P3	Lung Carcinoma	+	>	+	+	-
	A431	Epidermoid Carcinoma	+		-	ND	ND
	K562	Erythroleukemia	+		+	ND	ND
10	U937	Histiocytic Lymphoma	+	>	+	+	-
	804G(Rat)	Bladder Carcinoma	+		-	+	-
	3T3 (M)	Embryonic Fibroblast	+		-	+	-
	F9 (M)	Teratocarcinoma	-		+	+	-
	ES (M)	Embryonic Stem	-		+	+	-
15	ES (M)	(Differentiated)	+		+	ND	ND

Cells were analyzed by PCR amplification of α_6 and α_3 isoforms using the following human or mouse cells, with the cell sources indicated in parenthesis: pancreatic carcinoma: FG, SGR and 1320 Met cells (Dr. P. Meitner, Brown University); Panc-1 cells (ATCC CRL 1469); choriocarcinoma: JAR cells (ATCC HTB 144); JEG-3 cells (ATCC HTB 36); BeWo cells (ATCC CCL 98); colon carcinoma: LoVo cells (ATCC CCL 229); Colo 396 cells (Dr. T. Edgington, Scripps Clinic, La Jolla, CA); CaCo-2 cells (ATCC HTB 37); HT-29 cells (ATCC HTB 38); Hela cervical carcinoma cells (ATCC CCL 2); UCLA-P3 lung carcinoma cells (L. Walker, Scripps); A431 epidermoid carcinoma cells (ATCC CRL 1555); K562 erythroleukemia cells (ATCC CCL 243); U937 histiocytic lymphoma cells (ATCC CCL 1593); 8049 rat bladder carcinoma cells (J. Jones, Northwestern University, Evanston, IL); NIH/3T3 mouse embryo fibroblasts (ATCC CRL 1658); F9 mouse teratocarcinoma cells ATCC CRL 1720); ES mouse embryonic stem cells (E. Robertson, Columbia University, NY).

Table 1 illustrates the distinction of α_{6A} and α_{6B} , and α_{3A} and α_{3B} subunit-encoding mRNAs in human and mouse cultured cell lines. PCR amplification was performed on single-stranded cDNA generated from each cell type, using oligonucleotides specific for the α_6 or α_3 subunit, respectively. The (+) symbol represents the presence of subunit-specific amplification product in the tested sample, the (-) symbol represents its absence, and (ND) indicates that analysis was not conducted on that tissue type. The (>) is used when the α_{6A} subunit mRNA predominates over the α_{6B} subunit mRNA, and the (<) symbol is used when the α_{6B} subunit mRNA is the predominant species in the tissue.

By the same PCR assay, normal mouse lung, liver, spleen and cervix tissues were solely α_{6A} , brain, ovary and kidney were solely α_{6B} , while all other tissues tested contained both α_6 isoforms (Table 2).

TABLE 2

TISSUE	α_{6A}		α_{6B}	α_{3A}	α_{3B}
Muscle	+	>	+	+	-
Heart	+	>	+	-	+
Kidney	-		+	-	-
Liver	+		-	-	-
Brain	-		+	+	< +
Lung	+		-	+	-
Stomach	+	<	+	ND	ND
Intestine	+	<	+	ND	ND
Cervix	+		-	ND	ND
Submax	+		+	ND	ND
Ovary	-		+	+	-
Thymus	+	>	+	-	-
Spleen	+		-	-	-

Table 2 illustrates the distribution of α_{6A} and B and α_{3A} and B subunit-encoding mRNAs in mouse tissues. The symbols in Table 2 are the same as in Table 1.

Primary and nested PCR reactions were carried out on differentiated cell lines as described in Example 3. ES1 cells were allowed to differentiate over a period of 8-9 days in the absence of Leukemia Inhibitory Factor (LIF). The morphology of the differentiated cells was dramatically different from that of undifferentiated ES1 cells maintained in LIF. PCR amplification on cDNA from undifferentiated ES1 cells, using α_6 specific primers, produced the 380 bp fragment corresponding to the α_{6B} cytoplasmic sequence (Figure 7B, lane 1). However, similar amplification of cDNA from the differentiated cells produced two distinct fragments of 510 bp and 380 bp (Figure 7B, lane 2), shown by nucleotide sequencing to be the α_{6A} and α_{6B} isoforms, respectively.

b. In Situ Immunostaining of Tissues to Detect Tissue Distribution

Kidney biopsy materials were obtained by percutaneous needle biopsies using modified Vim-Silverman needles in patients with glomerulonephritis. A small portion of kidney biopsy materials were fixed with 4% paraformaldehyde for 4 hours at 4°C and embedded in paraffin using an automatic processor (Tissue-Tek^R Rotary Tissue Processor). The tissue was cut in 4 micron thickness with an AO rotary microtome, and deparafinized with xylene or HistoClear (Baxter) and rehydrated with graded alcohol. The rehydrated sections were washed with 0.1 M glycine in TBS (0.005 M Tris-HCl; 0.9% NaCl, pH 7.5) for 5 minutes, treated with 0.1% Triton X-100 for 2 min at room temperature (RT), and trypsinized (0.1% trypsin for 5 min at 37°C). Nonspecific binding sites were saturated by a

blocking solution (5% dry milk solids, 1% heat inactivated horse serum in TBS) for 30 minutes. Serially diluted primary antibodies [1 ug/ul of 33% saturated ammonium sulfate (SAS) cut of antisera 6844 and 382; 1:10 to 1:1000 dilutions in reagent diluent: i.e. 2.5% bovine serum albumin in TBS] and normal control (SAS cut of normal rabbit serum, 1ug/ul, 1:10 to 1:1000 dilution in reagent diluent) were incubated on sections in humidified chambers at room temperature for 1 hr. Tissue sections were further incubated with peroxidase conjugated goat anti-rabbit antibody (Jackson Immunology) in reagent diluent (1:200) for 30 minutes. After this, 0.02% AEC (3-Amino- α -ethylcarbozole, Aldrich) was applied for 30 minutes at room temperature. Each step was followed by 3-minute washes in 0.005 M Tris-HCl, 0.9% NaCl pH 7.5 (TBS wash). Washed tissue sections were counterstained with Mayor's hematoxylin for 30 sec, mounted in Gel Mount (Biomeda) and observed under a light microscope.

The results of the in situ immunostained kidney sections using the anti-peptide antisera specific for α_{6A} (6844) or specific for α_{6B} (382) are shown in Figure 9. Panel A shows anti- α_{6A} antibody molecules staining podocytes in the glomerular structure of the kidney but no staining in the tubules of the kidney. Panel B shows anti- α_{6B} antibody molecules staining the epithelial cells of the distal or collecting tubules of the kidney but not the glomerular cells.

Additional kidney samples were similarly analyzed and the results are shown in Table 3.

TABLE 3¹

Patient	Sera 6488		Sera 382	
	<u>G</u>	<u>T</u>	<u>G</u>	<u>T</u>
Normal	-	-	-	-
1	+	-	-	+
2	++	±	-	++
3	+++	-	-	+++
4	+++	-	-	++++
5	++++	-	-	++++

¹ "G" indicates the immunostaining pattern observed in glomerular epithelial cells of the kidney, whereas "T" indicates the immunostaining pattern observed in the tubular epithelial cells, where (-) indicates no staining and + to ++++ indicates increasing intensities of stain. Patients 1-5 are patients that all have glomerular nephritis clinically indicated as to require kidney biopsy.

The results in Table 3 indicate that in all patients exhibiting symptoms of kidney dysfunction a distinct staining pattern is observed, namely that antisera immunospecific for α_{6A} cytoplasmic domain (6488) reacts with glomerular cells and antisera immunospecific for 2246B cytoplasmic domain (382) reacts with the tubular epithelial cells.

These results show that antibodies immunoreactive with the cytoplasmic domain of α_{6B} are useful for distinguishing cell types in kidney sections, and particularly to identify distal and collecting tubular epithelial cells in patients having conditions of kidney dysfunction such as glomerular nephritis.

Although the present invention has now been described in terms of certain preferred embodiments,

and exemplified with respect thereto, one skilled in the art will readily appreciate that various modifications, changes, omissions and substitutions may be made without departing from the spirit thereof.

5 It is intended, therefore, that the present invention be limited solely by the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Tamura, Richard N.
Quaranta, Vito
- (ii) TITLE OF INVENTION: INTEGRIN ALPHA SUBUNIT CYTOPLASMIC
DOMAIN POLYPEPTIDES, ANTIBODIES AND METHODS
- (iii) NUMBER OF SEQUENCES: 16
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Office of Patent Counsel, TSRI
 - (B) STREET: 10666 North Torrey Pines Road, Mail Drop TPC8
 - (C) CITY: La Jolla
 - (D) STATE: California
 - (E) COUNTRY: United States
 - (F) ZIP: 92037
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: PCT/US92/
 - (B) FILING DATE: 04-MAY-1992
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 07/695,564
 - (B) FILING DATE: 03-MAY-1992
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Fitting, Thomas
 - (B) REGISTRATION NUMBER: 34,163
 - (C) REFERENCE/DOCKET NUMBER: BEC0010P
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 619-554-2937

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1073 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: unknown

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- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (ix) FEATURE:
 - (A) NAME/KEY: Domain
 - (B) LOCATION: 1012..1037
 - (D) OTHER INFORMATION: /label= TRANSMEMBRANE
/note= "The putative transmembrane region is encompassed by amino acids 1012-1037."
- (ix) FEATURE:
 - (A) NAME/KEY: Cleavage-site
 - (B) LOCATION: (23^24)
 - (D) OTHER INFORMATION: /note= "The mature protein is cleaved from the signal sequence between amino acids 23-24."
- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 223
 - (D) OTHER INFORMATION: /label= GLYCOSYLATION
/note= "Potential site of N-linked glycosylation."
- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 284
 - (D) OTHER INFORMATION: /label= GLYCOSYLATION
/note= "Potential site of N-linked glycosylation."
- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 370
 - (D) OTHER INFORMATION: /label= GLYCOSYLATION
/note= "Potential site of N-linked glycosylation."
- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 513
 - (D) OTHER INFORMATION: /label= GLYCOSYLATION
/note= "Potential site of N-linked glycosylation."
- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 731
 - (D) OTHER INFORMATION: /label= GLYCOSYLATION
/note= "Potential site of N-linked glycosylation."

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 748
- (D) OTHER INFORMATION: /label= GLYCOSYLATION
/note= "Potential site of N-linked glycosylation."

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 891
- (D) OTHER INFORMATION: /label= GLYCOSYLATION
/note= "Potential site of N-linked glycosylation."

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 927
- (D) OTHER INFORMATION: /label= GLYCOSYLATION
/note= "Potential site of N-linked glycosylation."

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 958
- (D) OTHER INFORMATION: /label= GLYCOSYLATION
/note= "Potential site of N-linked glycosylation."

(ix) FEATURE:

- (A) NAME/KEY: Binding-site
- (B) LOCATION: 230..238
- (D) OTHER INFORMATION: /note= "Represents a putative
cation binding domain."

(ix) FEATURE:

- (A) NAME/KEY: Binding-site
- (B) LOCATION: 324..332
- (D) OTHER INFORMATION: /note= "Represents a putative
cation binding domain."

(ix) FEATURE:

- (A) NAME/KEY: Binding-site
- (B) LOCATION: 386..394
- (D) OTHER INFORMATION: /note= "Represents a putative
cation binding domain."

(ix) FEATURE:

- (A) NAME/KEY: Binding-site
- (B) LOCATION: 441..449
- (D) OTHER INFORMATION: /note= "Represents a putative
cation binding domain."

(ix) FEATURE:

- (A) NAME/KEY: Domain
- (B) LOCATION: 1040..1044

(D) OTHER INFORMATION: /label= CYTOPLASMIC
 /note= "The cytoplasmic sequence, which is
 conserved in virtually all of the integrin ALPHA
 chains."

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 927..1073

(D) OTHER INFORMATION: /note= "The sequence encoded by the
 fragment of ALPHA 6A cDNA amplified using primers
 1156/1157."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met	Ala	Ala	Ala	Gly	Gln	Leu	Cys	Leu	Leu	Tyr	Leu	Ser	Ala	Gly	Leu	1	5	10	15
Leu	Ser	Arg	Leu	Gly	Ala	Ala	Phe	Asn	Leu	Asp	Thr	Arg	Glu	Asp	Asn	20	25	30	
Val	Ile	Arg	Lys	Tyr	Gly	Asp	Pro	Gly	Ser	Leu	Phe	Gly	Phe	Ser	Leu	35	40	45	
Ala	Met	His	Trp	Gln	Leu	Gln	Pro	Glu	Asp	Lys	Arg	Leu	Leu	Leu	Val	50	55	60	
Gly	Ala	Pro	Arg	Gly	Glu	Ala	Leu	Pro	Leu	Gln	Arg	Ala	Phe	Arg	Thr	65	70	75	80
Gly	Gly	Leu	Tyr	Ser	Cys	Asp	Ile	Thr	Ala	Arg	Gly	Pro	Cys	Thr	Arg	85	90	95	
Ile	Glu	Phe	Asp	Asn	Asp	Ala	Asp	Pro	Thr	Ser	Glu	Ser	Lys	Glu	Asp	100	105	110	
Gln	Trp	Met	Gly	Val	Thr	Val	Gln	Ser	Gln	Gly	Pro	Gly	Gly	Lys	Val	115	120	125	
Val	Thr	Cys	Ala	His	Arg	Tyr	Glu	Lys	Arg	Gln	His	Val	Asn	Thr	Lys	130	135	140	
Gln	Glu	Ser	Arg	Asp	Ile	Phe	Gly	Arg	Cys	Tyr	Val	Leu	Ser	Gln	Asn	145	150	155	160
Leu	Arg	Ile	Glu	Asp	Asp	Met	Asp	Gly	Gly	Asp	Trp	Ser	Phe	Cys	Asp	165	170	175	
Gly	Arg	Leu	Arg	Gly	His	Glu	Lys	Phe	Gly	Ser	Cys	Gln	Gln	Gly	Val	180	185	190	

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Ala Ala Thr Phe Thr Lys Asp Phe His Tyr Ile Val Phe Gly Ala Pro
 195 200 205
 Gly Thr Tyr Asn Trp Lys Gly Ile Val Arg Val Glu Gln Lys Asn Asn
 210 215 220
 Thr Phe Phe Asp Met Asn Ile Phe Glu Asp Gly Pro Tyr Glu Val Gly
 225 230 235 240
 Gly Glu Thr Glu His Asp Glu Ser Leu Val Pro Val Pro Ala Asn Ser
 245 250 255
 Tyr Leu Gly Phe Ser Leu Asp Ser Gly Lys Gly Ile Val Ser Lys Asp
 260 265 270
 Glu Ile Thr Phe Val Ser Gly Ala Pro Arg Ala Asn His Ser Gly Ala
 275 280 285
 Val Val Leu Leu Lys Arg Asp Met Lys Ser Ala His Leu Leu Pro Glu
 290 295 300
 His Ile Phe Asp Gly Glu Gly Leu Ala Ser Ser Phe Gly Tyr Asp Val
 305 310 315 320
 Ala Val Met Asp Leu Asn Lys Asp Gly Trp Gln Asp Ile Val Ile Gly
 325 330 335
 Ala Pro Gln Tyr Phe Asp Arg Asp Gly Glu Val Gly Gly Ala Val Tyr
 340 345 350
 Val Tyr Met Asn Gln Gln Gly Arg Trp Asn Asn Val Lys Pro Ile Arg
 355 360 365
 Leu Asn Gly Thr Lys Asp Ser Met Phe Gly Ile Ala Val Lys Asn Ile
 370 375 380
 Gly Asp Ile Asn Gln Asp Gly Tyr Pro Asp Ile Ala Val Gly Ala Pro
 385 390 395 400
 Tyr Asp Asp Leu Gly Lys Val Phe Ile Tyr His Gly Ser Ala Asn Gly
 405 410 415
 Ile Asn Thr Lys Pro Thr Gln Val Leu Lys Gly Ile Ser Pro Tyr Phe
 420 425 430
 Gly Tyr Ser Ile Ala Gly Asn Met Asp Leu Asp Arg Asn Ser Tyr Pro
 435 440 445
 Asp Val Ala Val Gly Ser Leu Ser Asp Ser Val Thr Ile Phe Arg Ser
 450 455 460

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Arg Pro Val Ile Asn Ile Gln Lys Thr Ile Thr Val Thr Pro Asn Arg
 465 470 475 480
 Ile Asp Leu Arg Gln Lys Thr Ala Cys Gly Ala Pro Ser Gly Ile Cys
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 Leu Gln Val Lys Ser Cys Phe Glu Tyr Thr Ala Asn Pro Ala Gly Tyr
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 Arg Lys Ser Gly Leu Ser Ser Arg Val Gln Phe Arg Asn Gln Gly Ser
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 Glu Pro Lys Tyr Thr Gln Glu Leu Thr Leu Lys Arg Gln Lys Gln Lys
 545 550 555 560
 Val Cys Met Glu Glu Thr Leu Trp Leu Gln Asp Asn Ile Arg Asp Lys
 565 570 575
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 580 585 590
 Ser Arg Arg Arg Val Asn Ser Leu Pro Glu Val Leu Pro Ile Leu Asn
 595 600 605
 Ser Asp Glu Pro Lys Thr Ala His Ile Asp Val His Phe Leu Lys Glu
 610 615 620
 Gly Cys Gly Asp Asp Asn Val Cys Asn Ser Asn Leu Lys Leu Glu Tyr
 625 630 635 640
 Lys Phe Cys Thr Arg Glu Gly Asn Gln Asp Lys Phe Ser Tyr Leu Pro
 645 650 655
 Ile Gln Lys Gly Val Pro Glu Leu Val Leu Lys Asp Gln Lys Asp Ile
 660 665 670
 Ala Leu Glu Ile Thr Val Thr Asn Ser Pro Ser Asn Pro Arg Asn Pro
 675 680 685
 Thr Lys Asp Gly Asp Asp Ala His Glu Ala Lys Leu Ile Ala Thr Phe
 690 695 700
 Pro Asp Thr Leu Thr Tyr Ser Ala Tyr Arg Glu Leu Arg Ala Phe Pro
 705 710 715 720
 Glu Lys Gln Leu Ser Cys Val Ala Asn Gln Asn Gly Ser Gln Ala Asp
 725 730 735

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Cys Glu Leu Gly Asn Pro Phe Lys Arg Asn Ser Asn Val Thr Phe Tyr
 740 745 750
 Leu Val Leu Ser Thr Thr Glu Val Thr Phe Asp Thr Pro Tyr Leu Asp
 755 760 765
 Ile Asn Leu Lys Leu Glu Thr Thr Ser Asn Gln Asp Asn Leu Ala Pro
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 Ile Thr Ala Lys Ala Lys Val Val Ile Glu Leu Leu Leu Ser Val Ser
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 Gly Val Ala Lys Pro Ser Gln Val Tyr Phe Gly Gly Thr Val Val Gly
 805 810 815
 Glu Gln Ala Met Lys Ser Glu Asp Glu Val Gly Ser Leu Ile Glu Tyr
 820 825 830
 Glu Phe Arg Val Ile Asn Leu Gly Lys Pro Leu Thr Asn Leu Gly Thr
 835 840 845
 Ala Thr Leu Asn Ile Gln Trp Pro Lys Glu Ile Ser Asn Gly Lys Trp
 850 855 860
 Leu Leu Tyr Leu Val Lys Val Glu Ser Lys Gly Leu Glu Lys Val Thr
 865 870 875 880
 Cys Glu Pro Gln Lys Glu Ile Asn Ser Leu Asn Leu Thr Glu Ser His
 885 890 895
 Asn Ser Arg Lys Lys Arg Glu Ile Thr Glu Lys Gln Ile Asp Asp Asn
 900 905 910
 Arg Lys Phe Ser Leu Phe Ala Glu Arg Lys Tyr Gln Thr Leu Asn Cys
 915 920 925
 Ser Val Asn Val Asn Cys Val Asn Ile Arg Cys Pro Leu Arg Gly Leu
 930 935 940
 Asp Ser Lys Ala Ser Leu Ile Leu Arg Ser Arg Leu Trp Asn Ser Thr
 945 950 955 960
 Phe Leu Glu Glu Tyr Ser Lys Leu Asn Tyr Leu Asp Ile Leu Met Arg
 965 970 975
 Ala Phe Ile Asp Val Thr Ala Ala Ala Glu Asn Ile Arg Leu Pro Asn
 980 985 990
 Ala Gly Thr Gln Val Arg Val Thr Val Phe Pro Ser Lys Thr Val Ala
 995 1000 1005

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Gln Tyr Ser Gly Val Pro Trp Trp Ile Ile Leu Val Ala Ile Leu Ala
1010 1015 1020

Gly Ile Leu Met Leu Ala Leu Leu Val Phe Ile Leu Trp Lys Cys Gly
1025 1030 1035 1040

Phe Phe Lys Arg Asn Lys Lys Asp His Tyr Asp Ala Thr Tyr His Lys
1045 1050 1055

Ala Glu Ile His Ala Gln Pro Ser Asp Lys Glu Arg Leu Thr Ser Asp
1060 1065 1070

Ala

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5629 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..5629
- (D) OTHER INFORMATION: /product= "Human ALPHA 6A"
/note= "SEQ ID NO: 2 is the 5629 base nucleotide
sequence of the human ALPHA 6A cDNA."

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 147..149
- (D) OTHER INFORMATION: /function= "Transcription
initiator"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 216..3365
- (D) OTHER INFORMATION: /product= "Human ALPHA 6A"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature

74

(B) LOCATION: 3264..3278
 (D) OTHER INFORMATION: /product= "The cytoplasmic sequence
 GFFKR."

(ix) FEATURE:

(A) NAME/KEY: misc_feature
 (B) LOCATION: 3261..3390
 (D) OTHER INFORMATION: /note= "The 130 nucleotide sequence
 present in SEQ ID NO: 2 but deleted from SEQ ID
 NO:4."

(ix) FEATURE:

(A) NAME/KEY: misc_feature
 (B) LOCATION: 2924..3455
 (D) OTHER INFORMATION: /note= "The sequence of the ALPHA
 6A cDNA amplified using primers 1156/1157."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGCGGACCGT CCCGGGGGTG GGGCCGGGCG CAGCGGCGAG AGGAGGCGAA GGTGGCTGCG	60
GTAGCAGCAG CGCGGCAGCC TCGGACCCAG CCCGGAGCGC AGGGCGGGCCG CTGCAGGTCC	120
CGGCTCCCTT CCCCCTGCGT CCGCCCATGG CCGCGCGCCG GCAGCTGTGC TTGCTCTACC	180
TGTCGGCGGG GCTCCTGTCC CGGCTCGGCG CAGCCTTCAA CTTGGACACT CGGGAGGACA	240
ACGTGATCCG GAAATATGGA GACCCCGGGA GCCTCTTCGG CTTCTCGCTG GCCATGCACT	300
GGCAACTGCA GCGCGAGGAC AAGCGGCTGT TGCTCGTGGG GGCCCCGCGC GGAGAAGCGC	360
TTCCACTGCA GAGAGCCTTC AGAACGGGAG GGCTGTACAG CTGCGACATC ACCGCCCCGGG	420
GGCCATGCAC GCGGATCGAG TTTGATAACG ATGCTGACCC CACGTCAGAA AGCAAGGAAG	480
ATCAGTGGAT GGGGGTCACC GTCCAGAGCC AAGGTCCAGG GGGCAAGGTC GTGACATGTG	540
CTCACCGATA TGA AAAAAGG CAGCATGTTA ATACGAAGCA GGAATCCCGA GACATCTTTG	600
GGCGGTGTTA TGTCTGAGT CAGAATCTCA GGATTGAAGA CGATATGGAT GGGGGAGATT	660
GGAGCTTTTG TGATGGGCGA TTGAGAGGCC ATGAGAAATT TGGCTCTTGC CAGCAAGGTG	720
TAGCAGCTAC TTTTACTAAA GACTTTCATT ACATTGTATT TGGAGCCCCG GGTACTTATA	780
ACTGGAAAGG GATTGTTTCG GTAGAGCAAA AGAATAACAC TTTTTTTGAC ATGAACATCT	840
TTGAAGATGG GCCTTATGAA GTTGGTGGAG AGACTGAGCA TGATGAAAGT CTCGTTCTCTG	900
TTCTTGCTAA CAGTTACTTA GGT TTTTCTT TGGACTCAGG GAAAGGTATT GTTTCTAAAG	960

ATGAGATCAC	TTTTGTATCT	GGTGCTCCCA	GAGCCAATCA	CAGTGGAGCC	GTGGTTTTGC	1020
TGAAGAGAGA	CATGAAGTCT	GCACATCTCC	TCCCTGAGCA	CATATTGAT	GGAGAAGGTC	1080
TGGCCTCTTC	ATTTGGCTAT	GATGTGGCGG	TGATGGACCT	CAACAAGGAT	GGGTGGCAAG	1140
ATATAGTTAT	TGGAGCCCCA	CAGTATTTTG	ATAGAGATGG	AGAAGTTGGA	GGTGCAGTGT	1200
ATGTCTACAT	GAACCAGCAA	GGCAGATGGA	ATAATGTGAA	GCCAATTCGT	CTTAATGCAA	1260
CCAAAGATTC	TATGTTTGGC	ATTGCAGTAA	AAAATATTGG	AGATATTAAT	CAAGATGGCT	1320
ACCCAGATAT	TGCAGTTGGA	GCTCCGTATG	ATGACTTGGG	AAAGGTTTTT	ATCTATCATG	1380
GATCTGCAAA	TGGAATAAAT	ACCAAACCAA	CACAGGTTCT	CAAGGGTATA	TCACCTTATT	1440
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CTAGTGGGAT	ATGCCTCCAG	GTTAAATCCT	GTTTGAATA	TACTGCTAAC	CCCGCTGGTT	1680
ATAATCCTTC	AATATCAATT	GTGGGCACAC	TTGAAGCTGA	AAAAGAAAGA	AGAAAATCTG	1740
GGCTATCCTC	AAGAGTTCAG	TTTCGAAACC	AAGGTTCTGA	GCCCAAATAT	ACTCAAGAAG	1800
TAACTCTGAA	GAGGCAGAAA	CAGAAAGTGT	GCATGGAGGA	AACCCTGTGG	CTACAGGATA	1860
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GCTCTCGTAG	GCGAGTGAAT	TCACTTCCAG	AAGTTCTTCC	AATTCTGAAT	TCAGATGAAC	1980
CCAAGACAGC	TCATATTGAT	GTTCACTTCT	TAAAAGAGGG	ATGTGGAGAC	GACAATGTAT	2040
GTAACAGCAA	CCTTAAACTA	GAATATAAAT	TTTGCACCCG	AGAAGGAAAT	CAAGACAAAT	2100
TTTCTTATTT	ACCAATTCAA	AAAGGTGTAC	CAGAACTAGT	TCTAAAAGAT	CAGAAGGATA	2160
TTGCTTTAGA	AATAACAGTG	ACAAACAGCC	CTTCCAACCC	AAGGAATCCC	ACAAAAGATG	2220
GCGATGACGC	CCATGAGGCT	AAACTGATTG	CAACGTTTCC	AGACACTTTA	ACCTATTCTG	2280
CATATAGAGA	ACTGAGGGCT	TTCCCTGAGA	AACAGTTGAG	TTGTGTTGCC	AACCAGAATG	2340
GCTCGCAAGC	TGACTGTGAG	CTCGGAAATC	CTTTTAAAAG	AAATTCAAAT	GTCACTTTTT	2400
ATTTGGTTTT	AAGTACAAC	GAAGTCACCT	TTGACACCCC	ATATCTGGAT	ATTAATCTGA	2460
AGTTAGAAAC	AACAAGCAAT	CAAGATAATT	TGGCTCCAAT	TACAGCTAAA	GCAAAAAGTGG	2520

TTATTGAACT GCTTTTATCG GTCTCGGGAG TTGCTAAACC TTCCCAGGTG TATTTTGGAG	2580
GTACAGTTGT TGGCGAGCAA GCTATGAAAT CTGAAGATGA AGTGGGAAGT TTAATAGAGT	2640
ATGAATTCAG GGTAATAAAC TTAGGTAAAC CTCTTACAAA CCTCGGCACA GCAACCTTGA	2700
ACATTCAGTG GCCAAAAGAA ATTAGCAATG GGAAATGGTT GCTTTATTTG GTGAAAGTAG	2760
AATGCAAAGG ATTGGAAAAG GTAACTTGTG AGCCACAAAA GGAGATAAAC TCCCTGAACC	2820
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ACAGAAAATT TTCTTTATTT GCTGAAAGAA AATACCAGAC TCTTAACTGT AGCGTGAACG	2940
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TGCGCTCGAG GTTATGGAAC AGCACATTTT TAGAGGAATA TTCCAACTG AACTACTTGG	3060
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GAGTACCTTG GTGGATCATC CTAGTGGCTA TTCTCGCTGG GATCTTGATG CTTGCTTTAT	3240
TAGTGTTTAT ACTATGGAAG TGTGGTTTCT TCAAGAGAAA TAAGAAAGAT CATTATGATG	3300
GCACATATCA CAAGGCTGAG ATCCATGCTC AGCCATCTGA TAAAGAGAGG CTTACTTCTG	3360
ATGCATAGTA TTGATCTACT TCTGTAATTG TGTGGATTCT TTAAACGCTC TAGGTACGAT	3420
GACAGTGTTT CCCGATACCA TGCTGTAAGG ATCGGGAAAG AAGAGCGAGA GATCAAAGAT	3480
GAAAAGTATA TTGATAACCT TGAAAAAAAAA CAGTGGATCA CAAAGTGGA CAGAAATGAA	3540
AGCTACTCAT AGCGGGGGCC TAAAAAAAAA AAAGCTTCAC AGTACCCAAA CTGCTTTTTT	3600
CAACTCAGAA ATTCAATTG GATTTAAAAG CCTGCTCAAT CCCTGAGGAC TGATTTCAGA	3660
GTGACTACAC ACAGTACGAA CCTACAGTTT TAACTGTGGA TATTGTTACG TAGCCTAAGG	3720
CTCCTGTTTT GCACAGCCAA ATTTAAAACT GTTGGAAATGG ATTTTCTTTT AACTGCCGTA	3780
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AGGGCCTGCC CAGTTGCACT CAGGTGACAT CCTCCAGATA GTGTAGCTGA GGAGGCACCT	3900
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TCACGTTAGC TGTCCCACAT CACAAGACTA TGCCATTGGG GTAGTTGTGT TTCAACGGAA	4020
AGTGCTGTCT TAAACTAAAT GTGCAATAGA AGGTGATGTT GCCATCCTAC CGTCTTTTCC	4080

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TCACTAAAAA	CACACAGGTG	CAACAGACTT	GAATGCTAGT	TATACTTATT	TGTATATGGT	4200
ATTTATTTTT	TGTTTTCTTT	ACAAACCATT	TTGTTATTGA	CTAACAGGCC	AAAGAGTCTC	4260
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TCAGTATGAC	CTAAATTATT	TACTGCAAAA	AGAAAATCTT	TATAAATGTA	CCAGAGAGAG	4380
TTGTTTTAAT	AACTTATCTA	TAAACTATAA	CCTCTCCTTC	ATGACAGCCT	CCACCCACAC	4440
ACCCAAAAGG	TTTAAGAAAT	AGAATTATAA	CTGTAAAGAT	GTTTATTTCA	GGCATTGGAT	4500
ATTTTTTACT	TTAGAAGCCT	GCATAATGTT	TCTGGATTTA	CATACTGTAA	CATTCAGGAA	4560
TTCTTGGAGA	AGATGGGTTT	ATTCAGTGAA	CTCTAGTGCG	GTTTACTCAC	TGCTGCAAAT	4620
ACTGTATATT	CAGGACTTGA	AAGAAATCGT	GAATGCCTAT	GGAAGTAGTG	GATCCAAACT	4680
GATCCAGTAT	AAGACTACTG	AATCTGCTAC	CAAAACAGTT	AATCAGTGAG	TCGAGTGTTT	4740
TATTTTTTGT	TTTGTTTCCT	CCCCTATCTG	TATTCCCAAA	AATTACTTTG	GGGCTAATTT	4800
AACAAGAACT	TTAAATTGTG	TTTTAATTGT	AAAAATGGCA	GGGGGTGGAA	TTATTACTCT	4860
ATACATTCAA	CAGAGACTGA	ATAGATATGA	AAGCTGATTT	TTTTTAATTA	CCATGCTTCA	4920
CAATGTTAAG	TTATATGGGG	AGCAACAGCA	AACAGGTGCT	AATTTGTTTT	GGATATAGTA	4980
TAAGCAGTGT	CTGTGTTTTG	AAAGAATAGA	ACACAGTTTG	TAGTGCCACT	GTTGTTTTGG	5040
GGGGGGCCTT	TTTTCTTTTT	CCGGAAAATC	CTTAAACCTT	AAGATACTAA	GGACGTTGTT	5100
TTGTTTGTAC	TTGGAATTCT	TAGTCACAAA	ATATATTTTG	TTTACAAAAA	TTTCTGTAAA	5160
ACAGGTTATA	ACAGTGTTTA	AAGTCTCAGT	TTCTTGCTTG	GGGAACTTGT	GTCCCTAATG	5220
TGTTAGATTG	CTAGATTGCT	AAGGAGCTGA	TACTTGACAG	TTTTTTAGAC	CTGTGTTACT	5280
AAAAAAAAGA	TGAATGTCGG	AAAAGGGTGT	TGGGAGGGTG	GTCAACAAAG	AAACAAAGAT	5340
GTTATGGTGT	TTAGACTTAT	GGTTGTAAAA	AATGTCATCT	CAAGTCAAGT	CACTGGTCTG	5400
TTTGCATTTG	ATACATTTTT	GTACTAACTA	GCATTGTAAA	ATTATTTTCAT	GATTAGAAAT	5460
TACCTGTGGA	TATTTGTATA	AAAGTGTGAA	ATAAATTTTT	TATAAAAGTG	TTCAATTGTT	5520
CGTAACACAG	CATTGTATAT	GTGAAGCAAA	CTCTAAAATT	ATAAATGACA	ACCTGAATTA	5580
TCTATTTTCAT	CAAAAAA	AAAAA	ACTTTATGGG	CACAACTGG		5629

(2) INFORMATION FOR SEQ ID NO:3:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1091 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (ix) FEATURE:
 (A) NAME/KEY: Region
 (B) LOCATION: 1..1091
 (D) OTHER INFORMATION: /note= "SEQ ID NO:3 is the 1091
 residue amino acid sequence of the human ALPHA 6B
 protein."
- (ix) FEATURE:
 (A) NAME/KEY: Region
 (B) LOCATION: 1..1044
 (D) OTHER INFORMATION: /note= "The sequence of SEQ ID NO:3
 is identical to SEQ ID NO:1 between amino acids 1
 and 1044."
- (ix) FEATURE:
 (A) NAME/KEY: Region
 (B) LOCATION: 927..1060
 (D) OTHER INFORMATION: /note= "Encompasses the sequence
 encoded by the fragment of ALPHA 6B cDNA amplified
 using primers 1156/1157."
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
- | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Ala | Ala | Gly | Gln | Leu | Cys | Leu | Leu | Tyr | Leu | Ser | Ala | Gly | Leu |
| 1 | | | | 5 | | | | 10 | | | | | | 15 | |
| Leu | Ser | Arg | Leu | Gly | Ala | Ala | Phe | Asn | Leu | Asp | Thr | Arg | Glu | Asp | Asn |
| | | | 20 | | | | 25 | | | | | | 30 | | |
| Val | Ile | Arg | Lys | Tyr | Gly | Asp | Pro | Gly | Ser | Leu | Phe | Gly | Phe | Ser | Leu |
| | | | 35 | | | | 40 | | | | | 45 | | | |
| Ala | Met | His | Trp | Gln | Leu | Gln | Pro | Glu | Asp | Lys | Arg | Leu | Leu | Leu | Val |
| | | | | | | 55 | | | | | | 60 | | | |

79

Gly Ala Pro Arg Gly Glu Ala Leu Pro Leu Gln Arg Ala Phe Arg Thr
 65 70 75 80
 Gly Gly Leu Tyr Ser Cys Asp Ile Thr Ala Arg Gly Pro Cys Thr Arg
 85 90 95
 Ile Glu Phe Asp Asn Asp Ala Asp Pro Thr Ser Glu Ser Lys Glu Asp
 100 105 110
 Gln Trp Met Gly Val Thr Val Gln Ser Gln Gly Pro Gly Gly Lys Val
 115 120 125
 Val Thr Cys Ala His Arg Tyr Glu Lys Arg Gln His Val Asn Thr Lys
 130 135 140
 Gln Glu Ser Arg Asp Ile Phe Gly Arg Cys Tyr Val Leu Ser Gln Asn
 145 150 155 160
 Leu Arg Ile Glu Asp Asp Met Asp Gly Gly Asp Trp Ser Phe Cys Asp
 165 170 175
 Gly Arg Leu Arg Gly His Glu Lys Phe Gly Ser Cys Gln Gln Gly Val
 180 185 190
 Ala Ala Thr Phe Thr Lys Asp Phe His Tyr Ile Val Phe Gly Ala Pro
 195 200 205
 Gly Thr Tyr Asn Trp Lys Gly Ile Val Arg Val Glu Gln Lys Asn Asn
 210 215 220
 Thr Phe Phe Asp Met Asn Ile Phe Glu Asp Gly Pro Tyr Glu Val Gly
 225 230 235 240
 Gly Glu Thr Glu His Asp Glu Ser Leu Val Pro Val Pro Ala Asn Ser
 245 250 255
 Tyr Leu Gly Phe Ser Leu Asp Ser Gly Lys Gly Ile Val Ser Lys Asp
 260 265 270
 Glu Ile Thr Phe Val Ser Gly Ala Pro Arg Ala Asn His Ser Gly Ala
 275 280 285
 Val Val Leu Leu Lys Arg Asp Met Lys Ser Ala His Leu Leu Pro Glu
 290 295 300
 His Ile Phe Asp Gly Glu Gly Leu Ala Ser Ser Phe Gly Tyr Asp Val
 305 310 315 320
 Ala Val Met Asp Leu Asn Lys Asp Gly Trp Gln Asp Ile Val Ile Gly
 325 330 335

80

Ala Pro Gln Tyr Phe Asp Arg Asp Gly Glu Val Gly Gly Ala Val Tyr
 340 345 350
 Val Tyr Met Asn Gln Gln Gly Arg Trp Asn Asn Val Lys Pro Ile Arg
 355 360 365
 Leu Asn Gly Thr Lys Asp Ser Met Phe Gly Ile Ala Val Lys Asn Ile
 370 375 380
 Gly Asp Ile Asn Gln Asp Gly Tyr Pro Asp Ile Ala Val Gly Ala Pro
 385 390 395 400
 Tyr Asp Asp Leu Gly Lys Val Phe Ile Tyr His Gly Ser Ala Asn Gly
 405 410 415
 Ile Asn Thr Lys Pro Thr Gln Val Leu Lys Gly Ile Ser Pro Tyr Phe
 420 425 430
 Gly Tyr Ser Ile Ala Gly Asn Met Asp Leu Asp Arg Asn Ser Tyr Pro
 435 440 445
 Asp Val Ala Val Gly Ser Leu Ser Asp Ser Val Thr Ile Phe Arg Ser
 450 455 460
 Arg Pro Val Ile Asn Ile Gln Lys Thr Ile Thr Val Thr Pro Asn Arg
 465 470 475 480
 Ile Asp Leu Arg Gln Lys Thr Ala Cys Gly Ala Pro Ser Gly Ile Cys
 485 490 495
 Leu Gln Val Lys Ser Cys Phe Glu Tyr Thr Ala Asn Pro Ala Gly Tyr
 500 505 510
 Asn Pro Ser Ile Ser Ile Val Gly Thr Leu Glu Ala Glu Lys Glu Arg
 515 520 525
 Arg Lys Ser Gly Leu Ser Ser Arg Val Gln Phe Arg Asn Gln Gly Ser
 530 535 540
 Glu Pro Lys Tyr Thr Gln Glu Leu Thr Leu Lys Arg Gln Lys Gln Lys
 545 550 555 560
 Val Cys Met Glu Glu Thr Leu Trp Leu Gln Asp Asn Ile Arg Asp Lys
 565 570 575
 Leu Arg Pro Ile Pro Ile Thr Ala Ser Val Glu Ile Gln Glu Pro Ser
 580 585 590
 Ser Arg Arg Arg Val Asn Ser Leu Pro Glu Val Leu Pro Ile Leu Asn
 595 600 605

81

Ser Asp Glu Pro Lys Thr Ala His Ile Asp Val His Phe Leu Lys Glu
 610 615 620

Gly Cys Gly Asp Asp Asn Val Cys Asn Ser Asn Leu Lys Leu Glu Tyr
 625 630 635 640

Lys Phe Cys Thr Arg Glu Gly Asn Gln Asp Lys Phe Ser Tyr Leu Pro
 645 650 655

Ile Gln Lys Gly Val Pro Glu Leu Val Leu Lys Asp Gln Lys Asp Ile
 660 665 670

Ala Leu Glu Ile Thr Val Thr Asn Ser Pro Ser Asn Pro Arg Asn Pro
 675 680 685

Thr Lys Asp Gly Asp Asp Ala His Glu Ala Lys Leu Ile Ala Thr Phe
 690 695 700

Pro Asp Thr Leu Thr Tyr Ser Ala Tyr Arg Glu Leu Arg Ala Phe Pro
 705 710 715 720

Glu Lys Gln Leu Ser Cys Val Ala Asn Gln Asn Gly Ser Gln Ala Asp
 725 730 735

Cys Glu Leu Gly Asn Pro Phe Lys Arg Asn Ser Asn Val Thr Phe Tyr
 740 745 750

Leu Val Leu Ser Thr Thr Glu Val Thr Phe Asp Thr Pro Tyr Leu Asp
 755 760 765

Ile Asn Leu Lys Leu Glu Thr Thr Ser Asn Gln Asp Asn Leu Ala Pro
 770 775 780

Ile Thr Ala Lys Ala Lys Val Val Ile Glu Leu Leu Leu Ser Val Ser
 785 790 795 800

Gly Val Ala Lys Pro Ser Gln Val Tyr Phe Gly Gly Thr Val Val Gly
 805 810 815

Glu Gln Ala Met Lys Ser Glu Asp Glu Val Gly Ser Leu Ile Glu Tyr
 820 825 830

Glu Phe Arg Val Ile Asn Leu Gly Lys Pro Leu Thr Asn Leu Gly Thr
 835 840 845

Ala Thr Leu Asn Ile Gln Trp Pro Lys Glu Ile Ser Asn Gly Lys Trp
 850 855 860

Leu Leu Tyr Leu Val Lys Val Glu Ser Lys Gly Leu Glu Lys Val Thr
 865 870 875 880

82

Cys Glu Pro Gln Lys Glu Ile Asn Ser Leu Asn Leu Thr Glu Ser His
 885 890 895
 Asn Ser Arg Lys Lys Arg Glu Ile Thr Glu Lys Gln Ile Asp Asp Asn
 900 905 910
 Arg Lys Phe Ser Leu Phe Ala Glu Arg Lys Tyr Gln Thr Leu Asn Cys
 915 920 925
 Ser Val Asn Val Asn Cys Val Asn Ile Arg Cys Pro Leu Arg Gly Leu
 930 935 940
 Asp Ser Lys Ala Ser Leu Ile Leu Arg Ser Arg Leu Trp Asn Ser Thr
 945 950 955 960
 Phe Leu Glu Glu Tyr Ser Lys Leu Asn Tyr Leu Asp Ile Leu Met Arg
 965 970 975
 Ala Phe Ile Asp Val Thr Ala Ala Ala Glu Asn Ile Arg Leu Pro Asn
 980 985 990
 Ala Gly Thr Gln Val Arg Val Thr Val Phe Pro Ser Lys Thr Val Ala
 995 1000 1005
 Gln Tyr Ser Gly Val Pro Trp Trp Ile Ile Leu Val Ala Ile Leu Ala
 1010 1015 1020
 Gly Ile Leu Met Leu Ala Leu Leu Val Phe Ile Leu Trp Lys Cys Gly
 1025 1030 1035 1040
 Phe Phe Lys Arg Ser Arg Tyr Asp Asp Ser Val Pro Arg Tyr His Ala
 1045 1050 1055
 Val Arg Ile Arg Lys Glu Glu Arg Glu Ile Lys Asp Glu Lys Tyr Ile
 1060 1065 1070
 Asp Asn Leu Glu Lys Lys Gln Trp Ile Thr Lys Trp Asn Arg Asn Glu
 1075 1080 1085
 Ser Tyr Ser
 1090

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5499 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..5499

(D) OTHER INFORMATION: /product= "Human ALPHA 6B"

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..3260

(D) OTHER INFORMATION: /note= "The sequence of SEQ ID NO:4 is identical to SEQ ID NO:2 between nucleotides 1 and 3260."

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 3261..5499

(D) OTHER INFORMATION: /note= "Nucleotides 3261-5499 of SEQ ID NO:4 are identical to nucleotides 3391-5629 of SEQ ID NO:2. SEQ ID NO:4 has a 130 nucleotide deletion in relation to SEQ ID NO:2."

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 2924..3325

(D) OTHER INFORMATION: /note= "Encompasses the sequence of the ALPHA 6B cDNA amplified using primers 1156/1157."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGCGACCGT CCCGGGGGTG GGGCCGGGCG CAGCGGCGAG AGGAGGCGAA GGTGGCTGCG	60
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CCGCTCCCCT CCGCGTGGCT CCGCCCATGG CCGCCGCCGG GCAGCTGTGC TTGCTCTACC	180
TGTCGGGGGG GCTCCTGTCC CGGCTCGGCG CAGCCTTCAA CTTGGACACT CGGGAGGACA	240
ACGTGATCCG GAAATATGGA GACCCCGGGA GCCTCTTCGG CTTCTCGCTG GCCATGCACT	300
GGCAACTGCA GCGCGAGGAC AAGCGGCTGT TGCTCGTGGG GGCCCCGCGC GGAGAAGCGC	360
TTCCACTGCA GAGAGCCTTC AGAACGGGAG GGCTGTACAG CTGCGACATC ACCGCCCGGG	420
GGCCATGCAC GCGGATCGAG TTTGATAACG ATGCTGACCC CACGTCAGAA AGCAAGGAAG	480

ATCAGTGGAT GGGGGTCACC GTCCAGAGCC AAGGTCCAGG GGGCAAGGTC GTGACATGTG 540
CTCACCGATA TGAAAAAAGG CAGCATGTTA ATACGAAGCA GGAATCCCGA GACATCTTTG 600
GGCGGTGTTA TGTCTGAGT CAGAATCTCA GGATTGAAGA CGATATGGAT GGGGGAGATT 660
GGAGCTTTTG TGATGGGCGA TTGAGAGGCC ATGAGAAATT TGGCTCTTGC CAGCAAGGTG 720
TAGCAGCTAC TTTTACTAAA GACTTTCATT ACATTGTATT TGGAGCCCCG GGTACTTATA 780
ACTGGAAGG GATTGTTCGT GTAGAGCAAA AGAATAACAC TTTTTTTGAC ATGAACATCT 840
TTGAAGATGG GCCTTATGAA GTTGGTGGAG AGACTGAGCA TGATGAAAGT CTCGTTCCCTG 900
TTCCTGCTAA CAGTTACTTA GGTTTTTCTT TGGACTCAGG GAAAGGTATT GTTTCTAAAG 960
ATGAGATCAC TTTTGTATCT GGTGCTCCCA GAGCCAATCA CAGTGGAGCC GTGGTTTTGC 1020
TGAAGAGAGA CATGAAGTCT GCACATCTCC TCCCTGAGCA CATATTCGAT GGAGAAGGTC 1080
TGGCCTCTTC ATTTGGCTAT GATGTGGCGG TGATGGACCT CAACAAGGAT GGGTGGCAAG 1140
ATATAGTTAT TGGAGCCCCA CAGTATTTTG ATAGAGATGG AGAAGTTGGA GGTGCAGTGT 1200
ATGTCTACAT GAACCAGCAA GGCAGATGGA ATAATGTGAA GCCAATTCGT CTTAATGGAA 1260
CCAAAGATTC TATGTTTGGC ATTGCAGTAA AAAATATTGG AGATATTAAT CAAGATGGCT 1320
ACCCAGATAT TGCAGTTGGA GCTCCGTATG ATGACTTGGG AAAGGTTTTT ATCTATCATG 1380
GATCTGCAAA TGGAATAAAT ACCAAACCAA CACAGGTCTT CAAGGGTATA TCACCTTATT 1440
TTGGATATTC AATTGCTGGA AACATGGACC TTGATCGAAA TTCCTACCCT GATGTTGCTG 1500
TTGGTTCCCT CTCAGATTCA GTAACATTTT TCAGATCCCG GCCTGTGATT AATATTCAGA 1560
AAACCATCAC AGTAACTCCT AACAGAATTG ACCTCCGCCA GAAAACAGCG TGTGGGGCGC 1620
CTAGTGGGAT ATGCCTCCAG GTTAAATCCT GTTTTGAATA TACTGCTAAC CCGGCTGGTT 1680
ATAATCCTTC AATATCAATT GTGGGCACAC TTGAAGCTGA AAAAGAAAAGA AGAAAAATCTG 1740
GGCTATCCTC AAGAGTTCAG TTTCGAAACC AAGGTCTGA GCCCAAATAT ACTCAAGAAC 1800
TAACTCTGAA GAGGCAGAAA CAGAAAGTGT GCATGGAGGA AACCTGTGG CTACAGGATA 1860
ATATCAGAGA TAAACTGCGT CCCATTCCCA TAACTGCCTC AGTGGAGATC CAAGAGCCAA 1920
GCTCTCGTAG GCGAGTGAAT TCACTTCCAG AAGTTCTTCC AATTCTGAAT TCAGATGAAC 1980
CCAAGACAGC TCATATTGAT GTTCACTTCT TAAAAGAGGG ATGTGGAGAC CACAATGTAT 2040

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TTTCTTATTT	ACCAATTCAA	AAAGGTGTAC	CAGAACTAGT	TCTAAAAGAT	CAGAAGGATA	2160
TTGCTTTAGA	AATAACAGTG	ACAAACAGCC	CTTCCAACCC	AAGGAATCCC	ACAAAAGATG	2220
GCGATGACGC	CCATGAGGCT	AAACTGATTG	CAACGTTTCC	AGACACTTTA	ACCTATTCTG	2280
CATATAGAGA	ACTGAGGGCT	TTCCCTGAGA	AACAGTTGAG	TTGTGTTGCC	AACCAGAATG	2340
GCTCGCAAGC	TGACTGTGAG	CTCGGAAATC	CTTTTAAAAG	AAATTCAAAT	GTCACTTTTT	2400
ATTTGGTTTT	AAGTACAACT	GAAGTCACCT	TTGACACCCC	ATATCTGGAT	ATTAATCTGA	2460
AGTTAGAAAC	AACAAGCAAT	CAAGATAATT	TGGCTCCAAT	TACAGCTAAA	GCAAAAGTGG	2520
TTATTGAACT	GCTTTTATCG	GTCTCGGGAG	TTGCTAAACC	TTCCCAGGTG	TATTTTGGAG	2580
GTACAGTTGT	TGGCGAGCAA	GCTATGAAAT	CTGAAGATGA	AGTGGGAAGT	TTAATAGAGT	2640
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ACATTCAGTG	GCCAAAAGAA	ATTAGCAATG	GGAAATGGTT	GCTTTATTTG	GTGAAAGTAG	2760
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ACAGAAAATT	TTCTTTATTT	GCTGAAAGAA	AATACCAGAC	TCTTAACTGT	AGCGTGAACG	2940
TGAACTGTGT	GAACATCAGA	TGCCCCGCTC	GGGGGCTGGA	CAGCAAGGCG	TCTCTTATTT	3000
TGCGCTCGAG	GTTATGGAAC	AGCACATTTT	TAGAGGAATA	TTCCAAACTG	AACTACTTGG	3060
ACATTCTCAT	GCGAGCCTTC	ATTGATGTGA	CTGCTGCTGC	CGAAAATATC	AGGCTGCCAA	3120
ATGCAGGCAC	TCAGGTTTGA	GTGACTGTGT	TTCCCTCAAA	GAAGTGTAGT	CAGTATTCTG	3180
GAGTACCTTG	GTGGATCATC	CTAGTGGCTA	TTCTGCGCTG	GATCTTGATG	CTTGCTTTAT	3240
TAGTGTTTAT	ACTATGGAAG	TGTGGATTCT	TTAAACGCTC	TAGGTACGAT	GACAGTGTTT	3300
CCGGATACCA	TGCTGTAAGG	ATCCGGAAAG	AAGAGCGAGA	GATCAAAGAT	GAAAAGTATA	3360
TTGATAACCT	TGAAAAAAAA	CAGTGGATCA	CAAAGTGGAA	CAGAAATGAA	AGCTACTCAT	3420
AGCGGGGGCC	TAAAAAAAAA	AAAGCTTCAC	AGTACCCAAA	CTGCTTTTTT	CAACTCAGAA	3480
ATTCAATTTG	GATTTAAAAG	CCTGCTCAAT	CCCTGAGGAC	TGATTTTACA	GTGACTACAC	3540
ACAGTACGAA	CCTACAGTTT	TAACTGTGGA	TATTGTTACG	TAGCCTAAGG	CTCCTGTTTT	3600

GCACAGCCAA	ATTTAAAACT	GTTGGAATGG	ATTTTTCTTT	AACTGCCGTA	ATTTAACTTT	3660
CTGGGTTGCC	TTTGTTTTTG	GCGTGGCTGA	CTTACATCAT	GTGTTGGGGA	AGGGCCTGCC	3720
CAGTTGCACT	CAGGTGAGAT	CCTCCAGATA	GTGTAGCTGA	GGAGGCACCT	ACACTCACCT	3780
GCACTAACAG	AGTGGCGGTC	CTAACCTCGG	GCCTGCTGGG	CAGACGTCCA	TCACGTTAGC	3840
TGTCCCACAT	CACAAGACTA	TGCCATTGGG	GTAGTTGTGT	TTCAACGGAA	AGTGCTGTCT	3900
TAAACTAAAT	GTGCAATAGA	AGGTGATGTT	GCCATCCTAC	CGTCTTTTCC	TGTTTCCTAG	3960
CTGTGTGAAT	ACCTGCTCAG	GTCAAATGCA	TACAAGTTTC	ATTCTCCCTT	TCACTAAAAA	4020
CACACAGGTG	CAACAGACTT	GAATGCTAGT	TATACTTATT	TGTATATGGT	ATTTATTTTT	4080
TGTTTTCTTT	ACAAACCATT	TTGTTATTGA	CTAACAGGCC	AAAGAGTCTC	CAGTTTACCC	4140
TTCAGGTTGG	TTTAATCAAT	CAGAATTAGA	ATTAGAGCAT	GGGAGGGTCA	TCACTATGAC	4200
CTAAATTATT	TACTGCAAAA	AGAAAATCTT	TATAAATGTA	CCAGAGAGAG	TTGTTTTAAT	4260
AACTTATCTA	TAAACTATAA	CCTCTCCTTC	ATGACAGCCT	CCACCCGACA	ACCCAAAAGG	4320
TTTAAGAAAT	AGAATTATAA	CTGTAAAGAT	GTTTATTTCA	GGCATTGGAT	ATTTTTTACT	4380
TTAGAAGCCT	GCATAATGTT	TCTGGATTTA	CATACTGTAA	CATTCAGGAA	TTCTTGAGAA	4440
AGATGGGTTT	ATTCAGTCAA	CTCTAGTGCG	GTTTACTCAC	TGCTGCAAAT	ACTGTATATT	4500
CAGGACTTCA	AAGAAATGGT	GAATGCCTAT	GGAAGTAGTG	GATCCAAACT	GATCCAGTAT	4560
AAGACTACTG	AATCTGCTAC	CAAAACAGTT	AATCAGTGAG	TCGAGTGTTT	TATTTTTTGT	4620
TTTGTTTCCT	CCCCTATCTG	TATTCCCAAA	AATTACTTTG	GGGCTAATTT	AACAAGAACT	4680
TTAAATTGTG	TTTAAATTGT	AAAAATGGCA	GGGGGTGGAA	TTATTACTCT	ATACATTCAA	4740
CAGAGACTGA	ATAGATATGA	AAGCTGATTT	TTTTTAATTA	CCATGCTTCA	CAATGTTAAG	4800
TTATATGGGG	AGCAACAGCA	AACAGGTGCT	AATTTGTTTT	GGATATAGTA	TAAGCAGTGT	4860
CTGTGTTTTG	AAAGAATAGA	ACACAGTTTG	TAGTGCCACT	GTTGTTTTTG	GGGGGGCTTT	4920
TTTTCTTTTT	CCGGAAAATC	CTTAAACCTT	AAGATACTAA	GGACGTTGTT	TTGGTTGTAC	4980
TTGGAATTCT	TAGTCACAAA	ATATATTTTG	TTTACAAAAA	TTTCTGTAAA	ACAGGTTATA	5040
ACAGTGTTTA	AAGTCTCAGT	TTCTTGCTTG	GGGAACCTGT	GTCCCTAATG	TGTTAGATTG	5100
CTAGATTGCT	AAGGAGCTGA	TACTTGACAG	TTTTTTAGAC	CTGTGTTACT	AAAAAAAAGA	5160

TGAATGTCGG AAAAGGGTGT TGGGAGGGTG GTCAACAAAG AAACAAAGAT GTTATGGTGT 5220
TTAGACTTAT GGTGTGTTAA AATGTCATCT CAAGTCAAGT CACTGGTCTG TTTGCATTTG 5280
ATACATTTTT GTACTAACTA GCATTGTAAA ATTATTTTCAT GATTAGAAAT TACCTGTGGA 5340
TATTTGTATA AAAGTGTGAA ATAAATTTTT TATAAAAGTG TTCATTGTTT CGTAACACAG 5400
CATTGTATAT GTGAAGCAAA CTCTAAAATT ATAAATGACA ACCTGAATTA TCTATTTTCAT 5460
CAAAAAAAAA AAAAAAAAAA ACTTTATGGG CACAACCTGG 5499

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 141 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..141
- (D) OTHER INFORMATION: /note= "The 141 amino acid sequence predicted from the nucleic acid product which results from amplification of the mouse ALPHA 6B cDNA with primers 1157/1156."

(ix) FEATURE:

- (A) NAME/KEY: Domain
- (B) LOCATION: 88..113
- (D) OTHER INFORMATION: /note= "The putative transmembrane domain."

(ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..120
- (D) OTHER INFORMATION: /note= "SEQ ID NO:5 is identical to SEQ ID NO:7 at amino acid position 1 through 120; the two sequences diverge at amino acid 121."

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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Thr Leu Asn Cys Ser Val Asn Val Arg Cys Val Asn Ile Arg Cys Pro
1           5           10           15
Leu Arg Gly Leu Asp Ser Lys Ala Ser Leu Val Leu Arg Ser Arg Leu
          20           25           30
Trp Asn Ser Thr Phe Leu Glu Glu Tyr Ser Lys Leu Asn Tyr Leu Asp
          35           40           45
Ile Leu Leu Arg Ala Ser Ile Asp Val Thr Ala Ala Ala Gln Asn Ile
50           55           60
Lys Leu Leu Thr Ala Gly Thr Gln Val Arg Val Thr Val Phe Pro Ser
65           70           75           80
Lys Thr Val Ala Gln Tyr Ser Gly Val Ala Trp Trp Ile Ile Leu Leu
          85           90           95
Ala Val Leu Ala Gly Ile Leu Met Leu Ala Leu Leu Val Phe Leu Leu
100          105          110
Trp Lys Cys Gly Phe Phe Lys Arg Ser Arg Tyr Asp Asp Ser Ile Pro
115          120          125
Arg Tyr His Ala Val Arg Ile Arg Lys Glu Glu Arg Glu
130          135          140

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 426 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..426
- (D) OTHER INFORMATION: /product= "Mouse ALPHA 6B amino acid sequence in SEQ ID NO:5."

(ix) FEATURE:

- (A) NAME/KEY: misc_feature

- (B) LOCATION: 262..337
(D) OTHER INFORMATION: /function= "Putative transmembrane region."

(ix) FEATURE:

- (A) NAME/KEY: misc feature
(B) LOCATION: (342^343)
(D) OTHER INFORMATION: /note= "SEQ ID NO:6 is identical to SEQ ID NO:8 except for 130 nucleotides present in SEQ ID NO:8 but deleted between nucleotides 342 and 343 of SEQ ID NO:6."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GACTCTTAAC TGTAGCGTGA ACGTGAGGTG TGTGAACATC AGGTGCCCAC TCGGAGGGCT	60
GGACAGCAAG GCCTCTCTCG TTCTTCGTTT CAGGTTGTGG AACAGCACAT TTCTAGAGGA	120
ATATTCCAAA CTGAACTACT TGGACATTCT CCTGAGGGCT TCCATAGATG TCACCGCTGC	180
TGCTGAGAAT ATCAAGCTCC TCACCGCCGG CACTCAGGTT CGAGTGACGG TGTTTCCCTC	240
AAAGACTGTA GCTCAGTATT CAGGAGTAGC TTGGTGGATC ATCCTCCTGG CTGTTCTTGC	300
CGGGATTCTG ATGCTGGCTC TATTAGTGTT TTTACTGTGG AAGTGTGGAT TCTTTAAGCG	360
CTCTAGGTAC GATGACAGCA TTCCCCGATA CCATGCGGTG CGGATCCGGA AAGAAGAGCG	420
AGAGAT	426

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 149 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Region
(B) LOCATION: 1..149
(D) OTHER INFORMATION: /note= "The 149 amino acid sequence predicted from the product which results from

90

amplification of the mouse ALPHA 6A cDNA with
primers 1157/1156."

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 1..120

(D) OTHER INFORMATION: /note= "SEQ ID NO:7 is identical to
SEQ ID NO:5 at amino acid positions 1 through 120;
the sequences diverge at amino acid 121."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Thr	Leu	Asn	Cys	Ser	Val	Asn	Val	Arg	Cys	Val	Asn	Ile	Arg	Cys	Pro	1	5	10	15
Leu	Arg	Gly	Leu	Asp	Ser	Lys	Ala	Ser	Leu	Val	Leu	Arg	Ser	Arg	Leu	20	25	30	
Trp	Asn	Ser	Thr	Phe	Leu	Glu	Glu	Tyr	Ser	Lys	Leu	Asn	Tyr	Leu	Asp	35	40	45	
Ile	Leu	Leu	Arg	Ala	Ser	Ile	Asp	Val	Thr	Ala	Ala	Ala	Gln	Asn	Ile	50	55	60	
Lys	Leu	Leu	Thr	Ala	Gly	Thr	Gln	Val	Arg	Val	Thr	Val	Phe	Pro	Ser	65	70	75	80
Lys	Thr	Val	Ala	Gln	Tyr	Ser	Gly	Val	Ala	Trp	Trp	Ile	Ile	Leu	Leu	85	90	95	
Ala	Val	Leu	Ala	Gly	Ile	Leu	Met	Leu	Ala	Leu	Leu	Val	Phe	Leu	Leu	100	105	110	
Trp	Lys	Cys	Gly	Phe	Phe	Lys	Arg	Asn	Lys	Lys	Asp	His	Tyr	Asp	Ala	115	120	125	
Thr	Tyr	His	Lys	Ala	Glu	Ile	His	Thr	Gln	Pro	Ser	Asp	Lys	Glu	Arg	130	135	140	
Leu	Thr	Ser	Asp	Ala												145			

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 556 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..556

(D) OTHER INFORMATION: /product= "Mouse ALPHA 6A amino acid sequence in SEQ ID NO:7."
/note= "SEQ ID NO:8 is the 556 base nucleotide sequence corresponding to the mouse ALPHA 6A amino acid sequence SEQ ID NO:7, plus the first 109 nucleotides in the 3' noncoding region."

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 342..472

(D) OTHER INFORMATION: /note= "SEQ ID NO:8 is identical to SEQ ID NO:6 except it has a 130 base insertion (nucleotides 342-472 of SEQ ID NO:8) between nucleotides 352 and 353 of SEQ ID NO:6."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GACTCTTAAC TGTAGCGTGA ACGTGAGGTG TGTGAACATC AGGTGCCCAC TCGGAGGGCT	60
GGACAGCAAG GCCTCTCTCG TTCTTCGTTT CAGGTTGTGG AACAGCACAT TTCTAGAGGA	120
ATATTCCAAA CTGAACTACT TGGACATTCT CCTGAGGGCT TCCATAGATG TCACCGCTGC	180
TGCTCAGAAT ATCAAGCTCC TCACCGCCGG CACTCAGGTT CGAGTGACGG TGTTTCCCTC	240
AAAGACTGTA GCTCAGTATT CAGGAGTAGC TTGGTGGATC ATCCTCCTGG CTGTTCTTGC	300
CGGGATTCTG ATGCTGGGCTC TATTAGTGTT TTTACTGTGG AAGTGTGGCT TCTTCAAGAG	360
AAATAAGAAA GATCATTACG ATGCCACCTA TCACAAGGCT GAGATCCATA CTCAGCCGTC	420
TGATAAAGAG AGGCTTACTT CCGATGCATA GTATTGATCT ACTTCATAA TTGTGTGGAT	480
TCTTTAAGCG CTCTAGGTAC GATGACAGCA TTCCCGATA CCATGCGGTG CGGATCCGGA	540
AAGAAGAGCG AGAGAT	556

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 153 amino acids

(B) TYPE: amino acid

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(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 1..153

(D) OTHER INFORMATION: /note= "SEQ ID NO:9 is the 153 amino acid sequence predicted from the product which results from amplification of the mouse ALPHA 3B cDNA with primers 2032/2033."

(ix) FEATURE:

(A) NAME/KEY: Domain

(B) LOCATION: 108..112

(D) OTHER INFORMATION: /note= "The cytoplasmic sequence CDFFK begins at amino acid position 108."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala	Arg	Cys	Val	Trp	Leu	Glu	Cys	Pro	Leu	Pro	Asp	Thr	Ser	Asn	Ile	1	5	10	15
Thr	Asn	Val	Thr	Val	Lys	Ala	Arg	Val	Trp	Asn	Ser	Thr	Phe	Ile	Glu	20	25	30	
Asp	Tyr	Lys	Asp	Phe	Asp	Arg	Val	Arg	Val	Asp	Gly	Trp	Ala	Thr	Leu	35	40	45	
Phe	Leu	Arg	Thr	Ser	Ile	Pro	Thr	Ile	Asn	Met	Glu	Asn	Lys	Thr	Thr	50	55	60	
Cys	Phe	Ser	Val	Asn	Ile	Asp	Ser	Lys	Leu	Leu	Glu	Glu	Leu	Pro	Ala	65	70	75	80
Glu	Ile	Glu	Leu	Trp	Leu	Val	Leu	Val	Ala	Val	Gly	Ala	Gly	Leu	Leu	85	90	95	
Leu	Leu	Gly	Leu	Ile	Ile	Ile	Leu	Leu	Trp	Lys	Cys	Asp	Phe	Phe	Lys	100	105	110	
Pro	Thr	Arg	Tyr	Tyr	Arg	Ile	Met	Pro	Lys	Tyr	His	Ala	Val	Arg	Ile	115	120	125	

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Arg Glu Glu Asp Arg Tyr Pro Pro Pro Gly Ser Thr Leu Pro Thr Lys
 130 135 140

Lys His Trp Val Thr Ser Trp Gln Ile
 145 150

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 463 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..463
- (D) OTHER INFORMATION: /product= "Mouse ALPHA 3B amino acid sequence in SEQ ID NO:9."
 /note= "SEQ ID NO:10 is the 463 base nucleotide sequence corresponding to the mouse ALPHA 3B amino acid sequence in SEQ ID NO:9."

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 324..338
- (D) OTHER INFORMATION: /product= "The cytoplasmic sequence CDFFK."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTGCCCCTG TGTGTGGCTG GAGTGCCCCC TTCCAGACAC CTCCAACATT ACCAATGTGA	60
CCGTGAAAGC ACGGGTGTGG AACAGCACCT TCATTGAGGA CTACAAAGAC TTTGACAGAG	120
TCAGGGTAGA TGGCTGGGCT ACCCTGTTCC TGAGAACCAG CATCCCTACC ATCAACATGG	180
AGAACAAGAC CACATGTTTC TCTGTGAACA TTGACTCAAA GCTGTTGGAG GAGCTGCCCCG	240
CTGAGATTGA GCTGTGGTTG GTGCTTGTGG CCGTGGGTGC TGGGTTGCTG CTGCTGGGGC	300
TCATCATCAT CCTCTTGTGG AAGTGTGACT TCTTAAGCC GACCCGCTAC TACCGGATTA	360
TGCCCAAGTA CCATGCAGTG CGTATCCGGG AGGAGGACCG CTACCCACCT CCAGGGAGCA	420

CGCTACCCAC CAAGAAGCAC TGGGTCACCA GCTGGCAGAT TCG

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(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /standard_name= "PCR PRIMER 1157"
/note= "Primer corresponds to bp 2918-2937 of the
ALPHA 6A cDNA sequence of SEQ ID NO:2."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GACTCTTAAC TGTAGCGTGA

20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /standard_name= "PCR PRIMER 1156"
/note= "The primer corresponds to the complement
of bp 3454-3473 of the APHA 6A cDNA sequence of
SEQ ID NO:2."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATCTCTCGCT CTTCTTCCG

20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..19
- (D) OTHER INFORMATION: /standard_name= "PCR PRIMER 1681"
/note= "The primer corresponds to bp 2942-2960 of
the ALPHA 6A cDNA sequence of SEQ ID NO:2."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAACTGTGTG AACATCAGA

19

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /standard_nam = "PCR PRIMER 2002"

/note= "The primer corresponds to the complement
of bp 3433-3452 of the ALPHA 6A cDNA sequence of
SEQ ID NO:2."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGCTTACAG CATGGTATCG

20

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..20
- (D) OTHER INFORMATION: /standard_name= "PCR PRIMER 2032"
/note= "The primer corresponds to the hamster
ALPHA 3A cDNA sequence of Tsuji et. al., J. Biol.
Chem., 265:7016-7021 (1990)."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AAGCGAAATC TGAGACTGTG

20

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..20

(D) OTHER INFORMATION: /standard_name= "PCR PRIMER 2033"
/note= "The primer corresponds to the hamster
ALPHA 3A cDNA sequence of Tsuji et al., J. Biol.
Chem., 265:7016-7021 (1990)."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTAGTATCGG TCCCGAATCT

20

What Is Claimed Is:

1. A polypeptide of about 24 to about 1091 amino acid residues in length having a sequence that includes the α_{6B} cytoplasmic domain sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091.
5
2. The polypeptide of claim 1 having a sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091.
3. The polypeptide of claim 1 having a sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.
10
4. A polypeptide having an amino acid residue sequence shown in SEQ ID NO 3 from residue 1 to residue 1091.
5. A polypeptide of about 21 to about 141 amino acid residues in length having a sequence that includes the α_{6B} cytoplasmic domain sequence shown in SEQ ID NO 5 from residue 121 to residue 141.
15
6. The polypeptide of claim 5 having a sequence shown in SEQ ID NO 5 from residue 121 to residue 141.
20
7. A polypeptide of about 41 to about 153 amino acid residues in length having a sequence that includes the α_{3B} cytoplasmic domain sequence shown in SEQ ID NO 9 from residue 113 to residue 153.
8. The polypeptide of claim 7 having a sequence shown in SEQ ID NO 9 from residue 113 to residue 153.
25
9. An antibody molecule that immunoreacts with the α_{6B} protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068-1091.
30
10. The antibody molecule of claim 9 wherein said antibody molecule is a monoclonal antibody molecule.
11. An antibody molecule that immunoreacts with the α_{6B} protein and with a polypeptide having an amino
35

acid residue sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.

12. The antibody molecule of claim 11 wherein said antibody molecule is a monoclonal antibody molecule.

13. An antibody molecule that immunoreacts with the α_{6B} protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141.

14. The antibody molecule of claim 13 wherein said antibody molecule is a monoclonal antibody molecule.

15. An antibody molecule that immunoreacts with the α_{3B} protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153.

16. The antibody molecule of claim 15 wherein said antibody molecule is a monoclonal antibody molecule.

17. A method for detecting the presence of antigen having the cytoplasmic domain of α_{6B} in a body sample comprising the steps of:

a) admixing the body sample with a composition containing antibody molecules that immunoreact with the α_{6B} protein and with a polypeptide consisting essentially of an amino acid residue sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091 to form an immunoreaction admixture;

b) maintaining said immunoreaction admixture under immunoreaction conditions for a time period sufficient for said antibody molecules to immunoreact with any α_{6B} present in said body sample and form an immunoreaction complex; and

c) detecting the presence of any immunoreaction complex formed in step (b) and thereby

detecting the presence of said antigen in said body sample.

18. The method of claim 17 wherein said detecting in step (c) comprises the steps of:

5 (i) admixing said immunoreaction product formed in step (b) with an indicating means to form a second reaction admixture;

10 (ii) maintaining said second reaction admixture for a time period sufficient for said indicating means to bind to the immunoreaction product formed in step (b) and form a second reaction product; and

15 (iii) determining the presence of said indicating means in said second reaction product, and thereby the presence of said immunoreaction product formed in step (b).

19. The method of claim 18 wherein said indicating means is a labeled antibody comprising an antibody having a label affixed thereto.

20 20. The method of claim 17 wherein said sample is a fluid sample, said admixing in step (a) includes admixing said body fluid sample and said antibody composition with a solid support comprising a solid matrix having affixed thereto a polypeptide having an amino acid residue sequence that includes an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091 such that said immunoreaction admixture is a competition immunoreaction admixture having a liquid phase and a solid phase, and said immunoreaction product formed in step (c) is in the solid phase.

30 21. The method of claim 20 wherein said antibody is a labeled antibody, having a label affixed to the antibody.

35 22. The method of claim 21 wherein said detecting in step (c) comprises determining the

presence of said label in the solid phase immunoreaction product, and thereby the presence of said immunoreaction product.

5 23. The method of claim 120 wherein said polypeptide has an amino acid residue sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.

10 24. The method of claim 17 wherein said sample is a fluid sample and said antibody molecules are affixed to a solid support such that said immunoreaction admixture is a competition immunoreaction admixture having a liquid phase and a solid phase, and said immunoreaction product formed in step (c) is in the solid phase.

15 25. The method of claim 24 wherein said admixing in step (b) includes admixing said body fluid sample and said solid-phase antibody composition with a polypeptide having an amino acid residue sequence that includes an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091 such that
20 said immunoreaction admixture is a competition immunoreaction admixture.

26. The method of claim 25 wherein said polypeptide is a labeled polypeptide, having a label affixed to the polypeptide.

25 27. A method for detecting the presence of antigen having the cytoplasmic domain of α_{68} in a body sample comprising the steps of:

30 a) admixing the body sample with a composition containing antibody molecules that immunoreact with the α_{68} protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141 to form an immunoreaction admixture;

35 b) maintaining said immunoreaction admixture under immunoreaction conditions for a time period sufficient for said antibody molecules to

immunoreact with any α_{6B} present in said body sample and form an immunoreaction complex; and

5 c) detecting the presence of any immunoreaction complex formed in step (b) and thereby detecting the presence of said antigen in said body sample.

28. A method for detecting the presence of antigen having the cytoplasmic domain of α_{3B} in a body sample comprising the steps of:

10 a) admixing the body sample with a composition containing antibody molecules that immunoreact with the α_{3B} protein and a polypeptide having an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153 to form an
15 immunoreaction admixture;

b) maintaining said immunoreaction admixture under immunoreaction conditions for a time period sufficient for said antibody molecules to immunoreact with any α_{3B} present in said body sample
20 and form an immunoreaction complex; and

c) detecting the presence of any immunoreaction complex formed in step (b) and thereby detect the presence of said antigen in said body sample.

25 29. A diagnostic system in kit form for assaying for the presence of α_{6B} subunit in a body sample, comprising a package containing, in an amount sufficient to perform at least one assay, an antibody composition comprising antibody molecules that
30 immunoreact with the α_{6B} protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 3 from residue 1045 to residue 1091.

35 30. The diagnostic system of claim 29 wherein said antibody is affixed to a solid matrix.

31. The diagnostic system of claim 29 that further includes a solid support comprised of a solid matrix having affixed thereto a polypeptide having an amino acid residue sequence that includes an amino acid residue sequence shown in SEQ ID NO 3 from residue 1068 to residue 1091.

32. The diagnostic system of claim 31 that further includes, in a separate package, labeled specific binding agent for signaling the presence of an immunoreaction product in the solid phase.

33. A diagnostic system in kit form for assaying for the presence of α_{6B} subunit in a body sample, comprising a package containing, in an amount sufficient to perform at least one assay, an antibody composition comprising antibody molecules that immunoreact with the α_{6B} protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 5 from residue 121 to residue 141.

34. A diagnostic system in kit form for assaying for the presence of α_{3B} subunit in a body sample, comprising separate packages containing, in an amount sufficient to perform at least one assay, an antibody composition comprising antibody molecules that immunoreact with the α_{3B} protein and with a polypeptide having an amino acid residue sequence shown in SEQ ID NO 9 from residue 113 to residue 153.

35. The diagnostic system of claim 32 that further includes a solid support comprised of a solid matrix having said antibody molecules affixed thereto.

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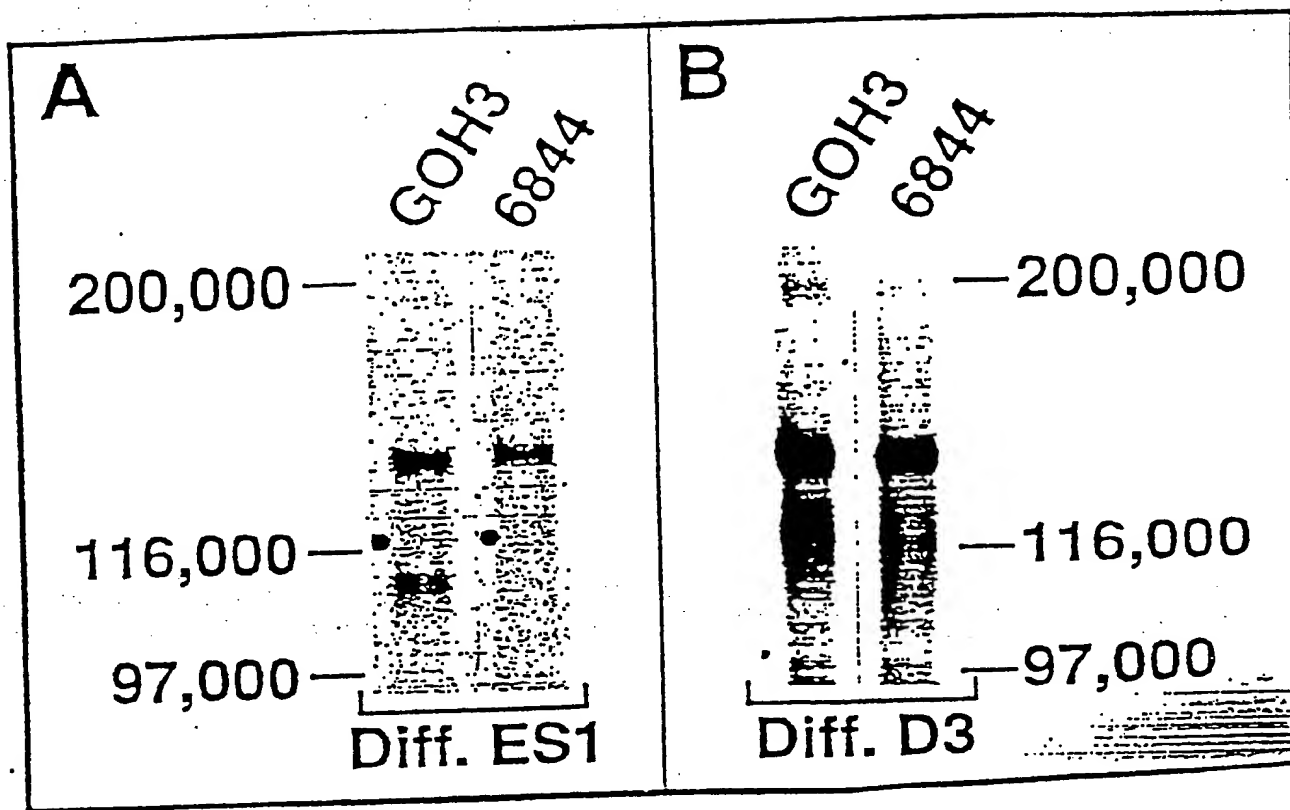


FIGURE 1

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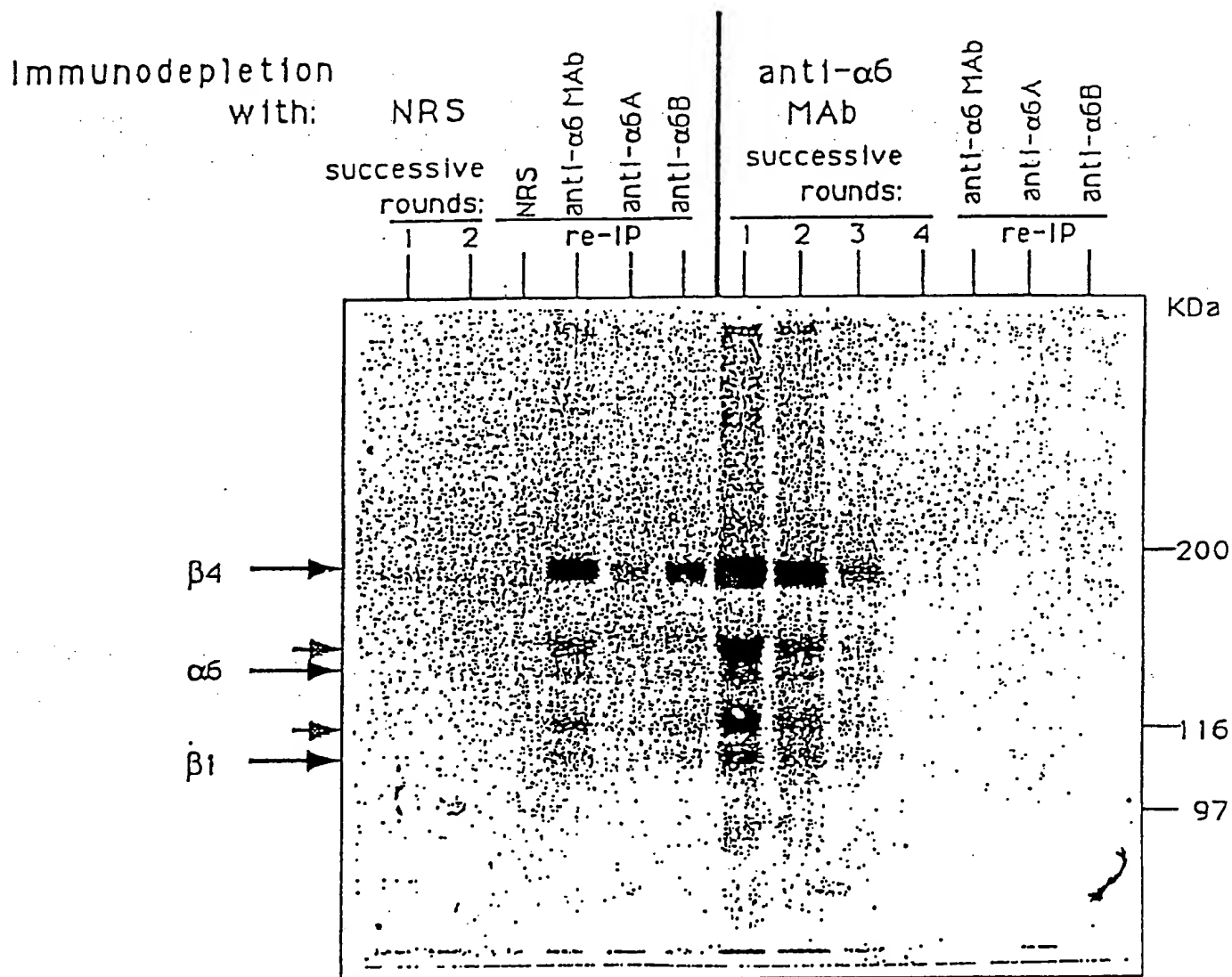


FIGURE 2

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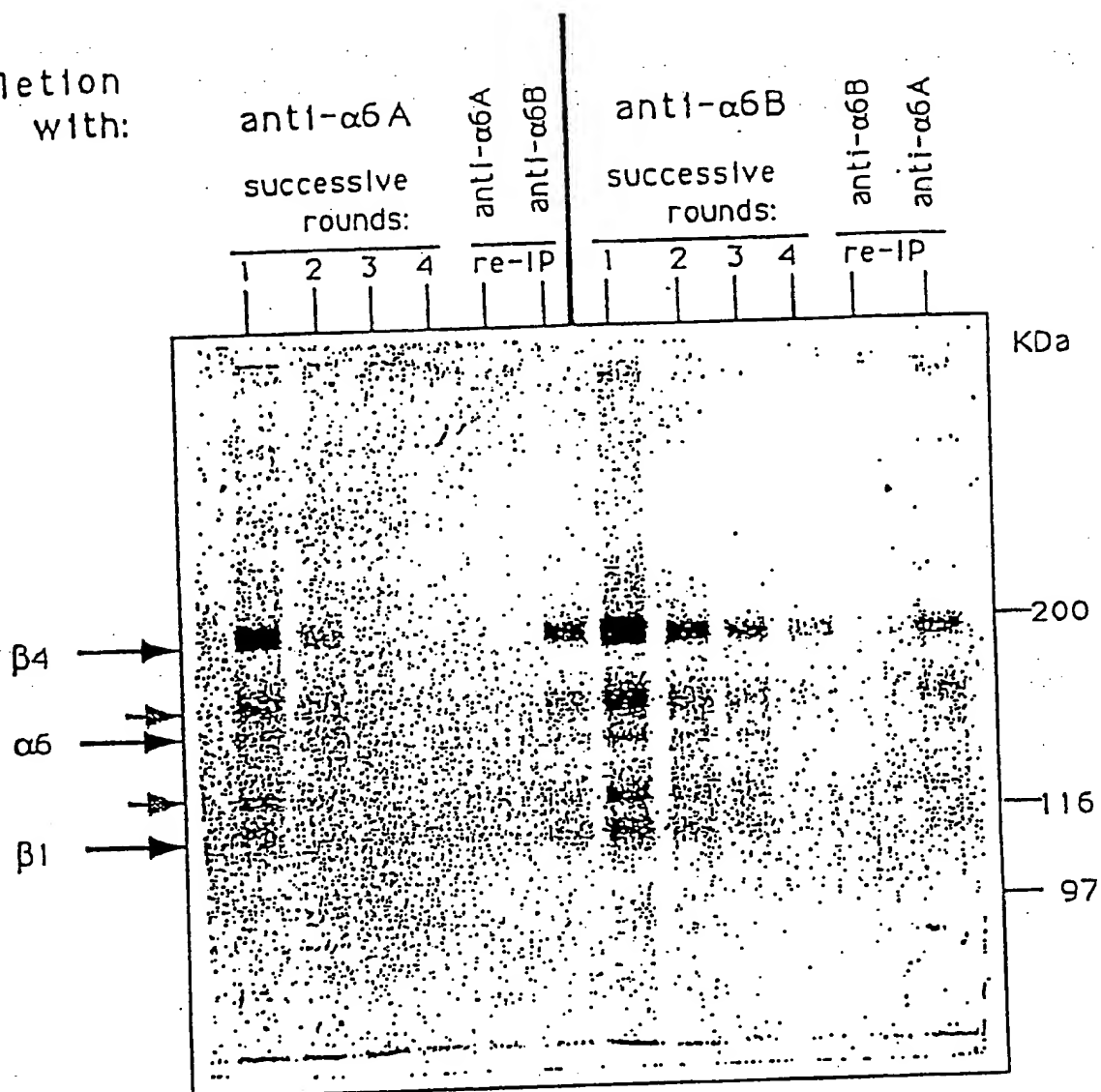
Immunodepletion
with:

FIGURE 3

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1 Kb pGFA FG JAR 0937 1 Kb

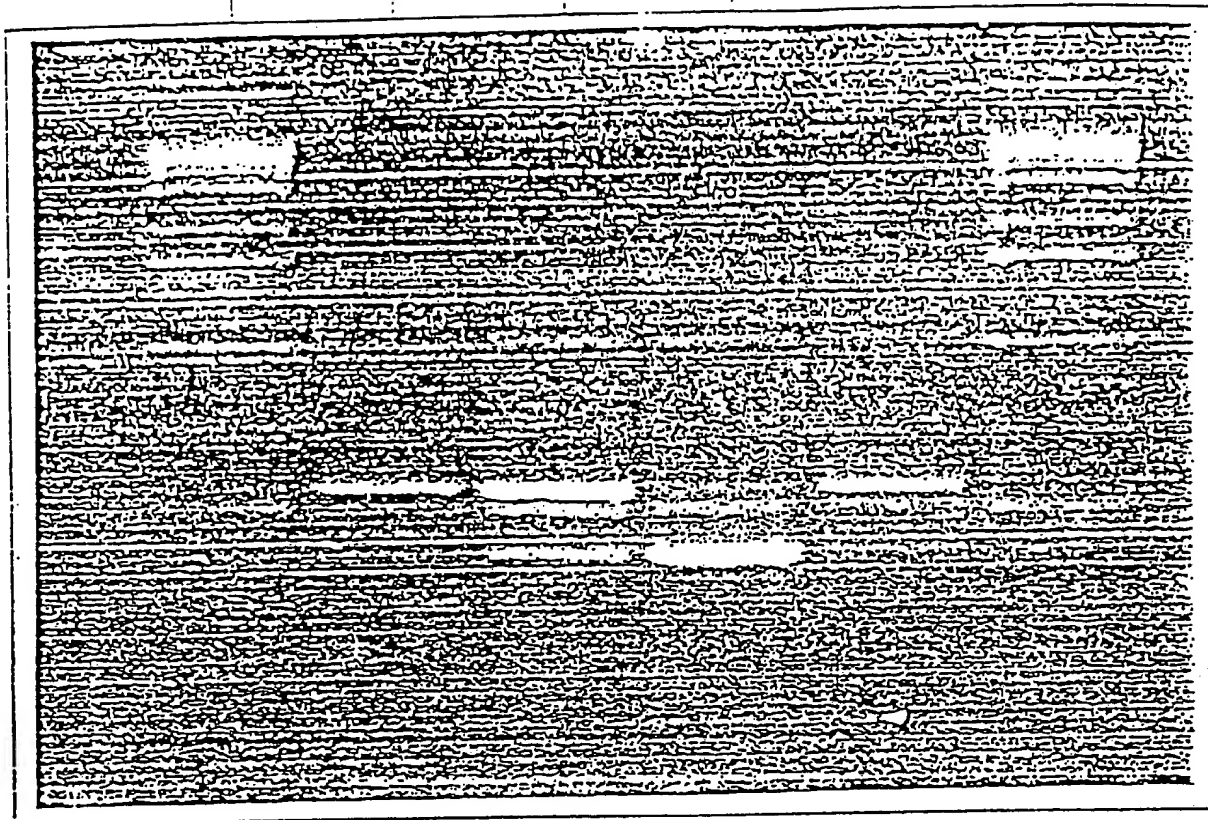


FIGURE 4

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 α 6A α 6B

2924	TAACGTAGCGTGAACGTGAACGTGTGTGAACATCAGATGCCCCGCTGCGGGGGCTGGACAG	2963
1	TAACGTAGCGTGAACGTGAACGTGTGTGAACATCAGATGCCCCGCTGCGGGGGCTGGACAG	60
2984	CAAGGCGTCTCTTATTTTGGCTCGAGGTTATGGAACAGCACATTTCTAGAGGAATATTC	3043
61	CAAGGCGTCTCTTATTTTGGCTCGAGGTTATGGAACAGCACATTTCTAGAGGAATATTC	120
3044	CAAACTGAACTACTTGGACATTCTCATGCGAGCCTTCATTGATGTGACTGCTGCTGCCGA	3103
121	CAAACTGAACTACTTGGACATTCTCATGCGAGCCTTCATTGATGTGACTGCTGCTGCCGA	180
3104	AAATATCAGGCTGCCAAATGCAGGCACTCAGGTTTCGAGTGACTGTGTTCCCTCAAAGAC	3163
181	AAATATCAGGCTGCCAAATGCAGGCACTCAGGTTTCGAGTGACTGTGTTCCCTCAAAGAC	240
3164	TGTAGCTCAGTATTCGGGAGTACCTTGGTGGATCATCCTAGTGGCTATTCTCGCTGGGAT	3223
241	TGTAGCTCAGTATTCGGGAGTACCTTGGTGGATCATCCTAGTGGCTATTCTCGCTGGGAT	300
3224	CTTGATGCTTGCTTTATTAGTGTTTATACTATGGAAGTGTTTCTTCAAGAGAAATAA	3283
301	CTTGATGCTTGCTTTATTAGTGTTTATACTATGGAAG.....	360
3284	GAAAGATCATTATGATGCCACATATCACAAGGCTGAGATCCATGCTCAGCCATCTGATAA	3343
361	420
3344	AGAGAGGCTTACTTCTGATGCATAGTATTGATCTACTTCTGTAATTGTGTGGATTCTTTA	3403
421TGTGGATTCTTTA	480
3404	AACGCTCTAGGTACGATGACAGTGTTCCCCGATACCATGCTGTAAGGATCCG	3455
481	AACGCTCTAGGTACGATGACAGTGTTCCCCGATACCATGCTGTAAGGATCCG	532

FIGURE 5

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1157 1681

2901 GCTGAAAGAAAATACCAGACTCTTAACTGTAGCGTGAACGTGAACGTGTGTGAACATCAGA 2960
A E R K Y Q T L N C S V N V N C V N I R

2961 TGCCCGCTGCGGGGGCTGGACAGCAAGGCGTCTCTTATTTTGGCGCTCGAGGTTATGGAAC 3020
C P L R G L D S K A S L I L R S R L W N

3021 AGCACATTTCTAGAGGAATATTTCCAACTGAACTACTTGGACATTCTCATGCCAGCCTTC 3080
S T F L E E Y S K L N Y L D I L M R A F

3081 ATTGATGTGACTGCTGCTGCCGAAAATATCAGGCTGCCAAATGCAGGCACCTCAGGTTCCA 3140
I D V T A A A E N I R L P N A G T Q V R

3141 GTGACTGTGTTTCCCTCAAAGACTGTAGCTCAGTATTCGGGAGTACCTTGGTGGATCATC 3200
V T V F P S K T V A Q Y S G V P W W I I

3201 CTAGTGGCTATTCTCGCTGGGATCTTGATGCTTGCTTTATTAGTGTATTACTATGGAAG 3260
I V A I L A G I L M L A L L V F I L W K

3261 TGTGGTTTCTTCAAGAGAAATAAGAAAGATCATTATGATGCCACATATCACAAGGCTGAG 3320
E G F F K R N K K D H Y D A T Y H K A E

3321 ATCCATGCTCAGCCATCTGATAAAGAGAGGCTTACTTCTGATGCATAGTATTGATCTACT 3380
I H A Q P S D K E R L T S D A 2002

3381 TCTGTAATTGCTGGATTCTTTAAACGCTCTAGGTACGATGACAGTGTTCCTCCCGATACCA 3440
E G F F K R S R Y D D S V P R Y H

3441 TGCTGTAAAGGATCCGGAAGAAGAGCGAGAGATCAAAGATGAAAAGTATATTGATAACCT 3500
A V R I R K E E R N E I K D E K Y I D N L

3501 TGAAAAAACAGTGGATCACAAAGTGGACAGAAATGAAAGCTACTCATAGCGGGGGGCC 3560
E K K Q W I T K W N R N E S Y S

3561 TAAAAAAGCTTCACAGTACCCAACTGCTTTTTTC 3600

FIGURE 6

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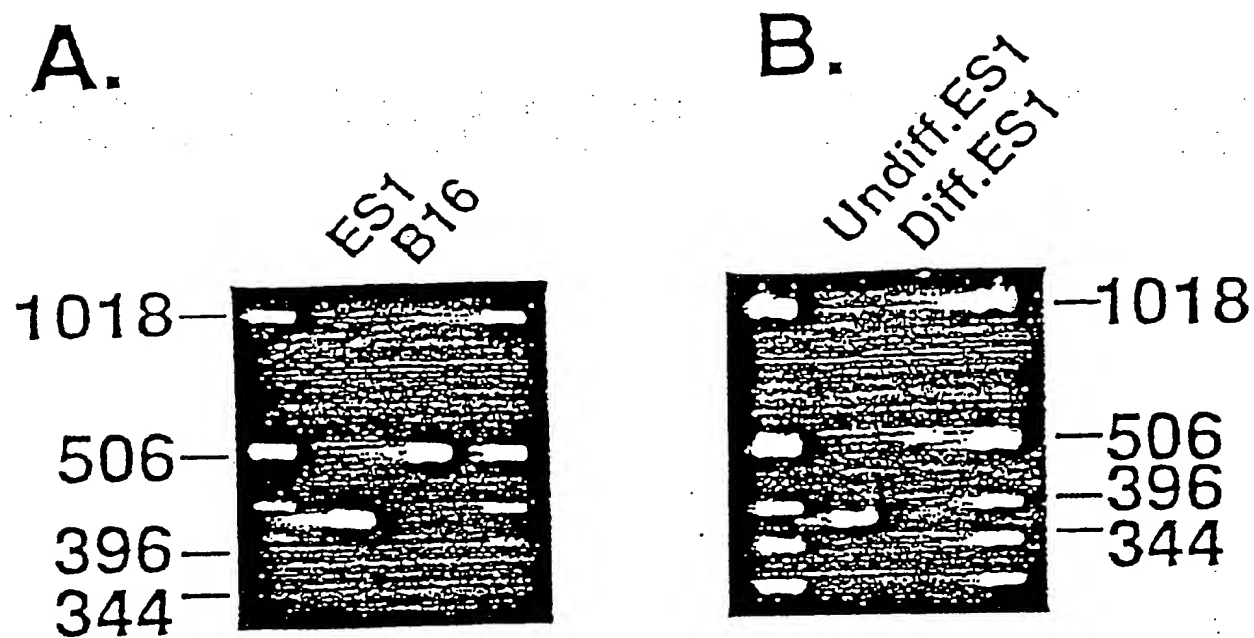


FIGURE 7

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1157
 gactcttaactgttagcgtgaacgtgaggtgtgtgaacatcaggtgcccactgcgagggct 2976
 gactcttaactgttagcgtgaacgtgaggtgtgtgaacatcaggtgcccactgcgagggct
 T L N C S V N V R C V N I R C P L R G L
 ggacagcaaggcctctctcgttcttcgttccaggttgtggaacagcacatttctagagga 3036
 ggacagcaaggcctctctcgttcttcgttccaggttgtggaacagcacatttctagagga
 D S K A S L V L R S R L W N S T F L E E
 atattccaaactgaactacttggacatttctcctgagggcttccatagatgtcaccgctgc 3096
 atattccaaactgaactacttggacatttctcctgagggcttccatagatgtcaccgctgc
 Y S K L N Y L D I L L R A S I D V T A A
 tgctcagaatatcaagctcctcaccgccggcactcaggttcgagtgacggtgtttccctc 3156
 tgctcagaatatcaagctcctcaccgccggcactcaggttcgagtgacggtgtttccctc
 A Q N I K L L T A G T Q V R V T V F P S
 aaagactgtagctcagtattcaggagtagcttgggtggatcatcctcctggctgttcttgc 3216
 aaagactgtagctcagtattcaggagtagcttgggtggatcatcctcctggctgttcttgc
 K T V A Q Y S G V A W W I I L L A V L A
 cgggattctgatgctggctctattagtggttttactgtggaa..... 3276
 cgggattctgatgctggctctattagtggttttactgtggaaagtgtgacttcttcaagag
 G I L M L A L L V F L L W K C G F F K R
 3336
 aaataagaaagatcattacgatgccacctatcacaaggctgagatccatactcagccgtc
 N K K D H Y D A T Y H K A E I H T Q P S
gtgtggat 3396
 tgataaagagagggcttacttccgatgcatagtattgatctacttccataattototoaat
 D K E R L T S D A * C G F
 tctttaagcgctctaggtacgatgacagcattccccgataccatgcggtgcggtatccgga 3456
 tctttaagcgctctaggtacgatgacagcattccccgataccatgcggtgcggtatccgga
 F K R S R Y D D S I P R Y H A V R I R K
 1156
 aagaagagcgagagat 3516
 aagaagagcgagagat
 E E R E

FIGURE 8

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A



B

FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/03527

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07K 7/10, 13/00, 15/28; G01N 33/68

US CL : 530/324, 325, 326, 350, 387.9, 388.22; 435/7.21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 325, 326, 350, 387.9, 388.22; 435/7.21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
chemical abstract service search terms: integrin, alpha subunit

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X.P Y	Eur. Journal of Biochemistry, Vol 199, issued July, 1991, R. Hogervorst et al., "Molecular cloning of the human $\alpha 6$ integrin subunit", pages 425-433. See abstract and Fig. 2.	1-4 9-12
Y	Journal of Biological Chemistry, Vol. 264, issued 15 April 1989, M. Hemler et al., "Association of the VLA $\alpha 6$ Subunit with a novel Protein, pages 6529-6535. See Fig. 5.	1-4, 9-12
Y	EMBO Journal, Vol. 8, no. 3, issued 1989 S. Kajiji et al., "A novel integrin ($\alpha E84$) from human epithelial cells suggests a fourth family of integrin adhesion receptors", pages 673-680. See abstract.	7, 8, 15, 16, 28, 34
Y	Journal of Biological Chemistry, Vol. 265, no. 12, issued 25 April 1990,, Tsuji et al. "Characterization through cDNA Cloning of Galactoprotein b3 (Gap b3), a Cell Surface Membrane Glycoprotein Showing Enhanced Expression on Oncogenic Transformation", pages 7016-7021. See abstract.	7, 8, 15, 16, 28, 34

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

21 July 1992

Date of mailing of the international search report

29 JUL 1992

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/03527

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Biological Chemistry, vol. 263, no. 16, issued 05 June 1988, M. Hemler et al., "Multiple Very Late Antigen (VLA) Heterodimers on Platelets", pages 7660-7665, see page 7660 abstract and column 2, and Table I.	1-6, 9-14, 17-27, 29-33, 35
A	Cell Differentiation and Development, Vol., 32, issued 1990, V. Quaranta, "Epithelial Integrins", pages 361-366, see entire document.	1-35